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14. ABSTRACT Optimal early detection and prevention strategies for breast cancer are predicated on our ability to identify individuals at significantly increased risk for this disease. The purpose of this Center was to bring molecular risk prediction for breast cancer into the clinical arena. The three areas of scientific inquiry included:(i) Establishment of a tissue repository of benign breast disease; (ii) Assessment of potential biomarkers of risk in this tissue set and (iii) Discovery of new, potentially relevant biomarkers of risk. We developed a cohort of 9,376 women, 758 (8%) of whom have been diagnosed with breast cancer since the time of their benign biopsy. We established our tissue repository of benign breast tissue and have collected the subsequent breast cancer tissue. We assessed the significance of benign histology in predicting the risk of future breast cancer, examining in detail the role of proliferative disease, atypia, papillomas, radial scars and involution. We explored the link between centrosome amplification, COX-2 expression, ER, Ki67 and breast cancer outcomes. Lastly, we characterized the histopathology in a cohort of African-American women that we established with Wayne State University.				
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**DAMD 17-02-1-0473**  
**Benign Breast Disease: Toward Molecular Predictor of Breast Cancer Risk**  
**Final Report - July 2009**

The purpose of the Center of Excellence was to bring molecular risk prediction for breast cancer into the clinical arena. The three main areas of scientific activity within this proposed Center were:

- the establishment of a large tissue repository from a retrospective cohort of women with benign breast disease (BBD) (1967-91) with complete and long-term clinical follow-up to identify those who developed breast cancer (cases) and those who did not (controls);
- the application of potential biomarkers of risk to this archival tissue set;
- the discovery of new, potentially relevant biomarkers of risk in fresh and frozen specimens of BBD.

We received our notice of funding in December of 2001. May 2002 the funding was released for the non-human subjects portion of the grant. February 2003 DOD approval was received for all portions of the grant. In 2007 we requested and received a no cost extension. The no-cost extension was expanded to the 2008 fiscal year to continue our work with Wayne State in African-American women. The Center included a multi-institutional team [(Mayo Clinic; UCSF; Wayne State)] of basic scientists, pathologists, epidemiologists, clinicians, statisticians, and advocates.

## **I. Establish a retrospective cohort of BBD and a nested case-control study**

### **A. *Development of study cohort***

We completed the identification of our study cohort, updated the cohort through use of a mailed questionnaire and chart abstraction, and obtained tissue from Mayo tissue registry and outside institutions. Our approach for each of these tasks and results are as follows:

**1. Identification of cohort.** Female patients who had a breast biopsy performed between January 1, 1967 and December 31, 1991 at the Mayo Clinic in Rochester, Minnesota (MCR) were identified using the resources of the Division of Medical Information (MIR). These include the surgical index, pathology index, medical index and tissue registry.

a. **Surgical Index.** For every surgical procedure performed at the MCR, MIR personnel review the surgical and pathology notes for the type of surgical procedures and diagnosis. The diagnostic data are coded using ICD-9 criteria and entered into a computer database referred to as the Surgical Index. We used the surgical index breast codes to begin our search

b. **Pathology Index.** The Pathology Index, a computerized database maintained by the Mayo Clinic Department of Laboratory Medicine and Pathology since 1985 contains all histologic findings for every surgery performed at the Mayo Clinic. This database was

searched to determine the histologic findings associated with each breast biopsy. The histologic findings of all breast biopsies performed prior to 1985 were abstracted by a trained nurse from pre-1985 Pathology Index maintained on index cards by month and year of procedure. For each woman, the histology from their first breast biopsy was reviewed. If the biopsy indicated a malignancy or was performed as part of a prophylactic mastectomy, the patient was not considered eligible for the study.

c. Medical Index and Tumor Registry. The next step in the screening process used two additional databases, the Medical Index and the Tumor Registry. MIR personnel maintain the Medical Index and Tumor Registry. Since 1975, the Medical Index has electronically captured the diagnostic findings of each patient's inpatient and outpatient episode of care. The Tumor Registry, begun in 1971, contains the diagnostic findings of any malignancy and routine follow-up of patients with a cancer diagnosis.

d. Cohort details. We identified 12,132 women, age 18 to 85, who had a surgical excision of a benign breast lesion during the 25 year period from January 1, 1967 through December 31, 1991. The median follow-up was 15 years. We excluded 1,047 women who had: a diagnosis of breast cancer or lobular carcinoma in situ at, before, or within six months of their breast biopsy; unilateral or bilateral mastectomy or breast reduction at or before their biopsy; or those who refused the use of their medical records for research. Of the remaining 11,085 women, 1,053 women had no follow-up information after their breast biopsy leaving 10,032 women. Another 945 women had unusable or unavailable biopsy tissue of the benign lesion. The remaining group of 9,087 women comprised our study cohort. A comparison of the 10,032 women who met study criteria and 9,087 women with available tissue show no significant difference in their relative risk of breast cancer (1.59 and 1.56, respectively). 7,260 women were alive at study entry, 1,827 were deceased.

#### ***B. Participant follow-up***

A study specific questionnaire was mailed to all eligible participants or, in the case of those who were deceased, their next-of-kin. Clinical-epidemiologic measures available from the questionnaires include: age at menarche; age when first child born; number of children; number of children breastfed more than one month; blood relatives with any cancer (degree and maternal/paternal) and ages; menopausal status; reason for menopause; oophorectomy (unilateral, bilateral; if yes, age at); use of birth control/oral contraceptives; if yes, ages taken and type; use of tamoxifen or raloxifene, year started and length of time taken; radiation to chest, if yes, age; number of breast biopsies; indication for breast biopsy; frequency of mammograms since breast biopsy; year of last routine mammogram; breast reduction, if yes, age; breast removal, reason, age, side; diagnosis of breast cancer, if yes, age, chemotherapy (before and/or after surgery), radiation (before/or after surgery), recurrence (date of, location); diagnosis of cancer anywhere other than breast, if yes, age diagnosed; height; weight (current and at age 18); race/ethnicity; use of non-steroidal anti-inflammatory drugs (specific drug, average days per month, average number of pills taken on days used, total number of years taken, age when taken); use of specific supplements (multivitamins, vitamin A, beta carotene, vitamin C, vitamin B, folic acid, vitamin E, calcium, selenium, zinc, garlic, fish oil, soy, black cohosh, shark cartilage); alcohol use, if ever used, age began to use regularly, current use, average consumption; physical activity and its frequency both during most of

adult life and currently. Questionnaire information was obtained on 5,619 (62%) of the women. Of these 604 (11%) were completed by next-of-kin.

***C. Validate reported breast cancers***

To validate reported breast cancers, charts were obtained for women diagnosed at Mayo Clinic and pertinent records were requested from outside institutions for women diagnosed outside Mayo Clinic. Prior to contacting outside institutions, women were asked to sign a consent form, giving researchers permission to access their outside medical records and tissue, as well as to identify the institution and physician providing care at the time of their cancer diagnosis. In the case of the deceased, next-of-kin were asked to give permission to obtain cancer related records. Once records were received, a trained nurse abstractor abstracted pertinent cancer information into database screens.

***D. Match breast cancer cases to controls***

For some studies, especially the biomarker studies, we used nested case:control series within our large cohort. To identify the case:control series, we randomly selected cases who developed breast cancer from the cohort, stratified by five-year categories of year of benign biopsy to represent the entire spectrum of the cohort. We individually matched two controls to each case based on age and year of benign biopsy.

***E. Test sets for preliminary evaluation of biomarkers***

**1. Establishment of laboratory procedures.** We tested a variety of procedures with a “technical set” of benign breast tissue from women not eligible for our study (e.g. those who had a benign biopsy after a previous cancer). We put the slides in long-term cold room storage to ascertain that labels would continue to adhere and the selected antibody dilution continued to work under those conditions. We then stained slides from the samples in the technical set that had been cold-stored for 3 to 6 months. The staining quality was assessed as “excellent” by our study pathologist.

**2. Sets for evaluation of biomarkers.**

a. Discovery set. A subset of approximately 125 cases and their two closest controls (matched on age at biopsy and year of biopsy) was chosen from the entire study period, 1967 to 1991, to serve as a test set. This set was used to ascertain a point and interval estimate of the prevalence of a candidate marker among the cases, as well as the risk of breast cancer among those with the candidate marker relative to those without the marker. The first step in constructing this test set was to determine the proportion of cases that occurred in each calendar year (1967 – 1991). The number of cases to be included in the test set for a particular year was that percentage of 125. Once the number to be selected from a particular calendar year was determined, that number was randomly selected from among the cases in that calendar year.

b. Atypia set. Given the high-risk status of women with atypia, we also focused on the atypia group (N = 336) to test the significance of biomarkers in them specifically.

## **II. Examine the association between select biomarkers in the benign specimens and subsequent risk of breast cancer**

### ***A. Obtain tissue***

**1. Benign breast tissue.** Archived benign breast tissue slides and paraffin blocks were obtained from Mayo Clinic Tissue Registry for the study cohort. For the first 25 years of the cohort (1967-1991), 838 women could not be included because their benign tissue was not available. Of those, 616 (73.5%) had their biopsies between 1967-1971. The reason for the large number of missing samples in the 1967-71 time period is that all benign tissue was not routinely preserved during that time. That policy was changed at Mayo and since 1977,  $\geq 95\%$  of the BBD samples were available. Of note, for the entire 1967-91 group, the relative risk of breast cancer did not differ significantly between the eligible women and the women for whom we had benign tissue, who made up the study cohort (1.59 and 1.56, respectively).

**2. Cancer tissue.** We were successful in obtaining cancer blocks on 407/427 (95%) of the women diagnosed at Mayo Clinic and 194/331 (59%) diagnosed outside of the Mayo Clinic. We have slides on an additional 5 (1%) of the women diagnosed at Mayo Clinic and 40 (12%) diagnosed outside of Mayo Clinic. Thus, altogether we have either slides or blocks from the breast cancers for 646 (85%) of the 758 women. No tissue has been obtained for 112/758 (15%) of women diagnosed with cancer. Of these women, we did not request permission on 40/112 (35%) as they or their next-of-kin did not complete the questionnaire. Additionally, 38/112 (34%) of these women or their next-of-kin did not grant permission for their tissue to be released to us. No tissue is available for the remaining 34 women.

### ***B. Characterize benign histology***

**1. Overall impression and risk.** In 2005, we published the first manuscript based on our Center of Excellence work. At that time, we had identified 707 breast cancers in our cohort of 9,087 women and examined the relative risk of breast cancer based on histology and family history. The overall relative risk of breast cancer for the cohort was 1.56 (95% CI 1.45 – 1.68), and this increased risk persisted for at least 25 years after biopsy. The relative risk associated with atypia was 4.24 (95% CI 3.25-5.41), for proliferative changes without atypia it was 1.88 (95% CI 1.66-2.12), and for non-proliferative lesions it was 1.27 (95% CI 1.15-1.41). Family history was a significant risk factor independent of histology. Women with a strong family history had a relative risk of 1.93 (95% CI .58-2.32) for no family history it was 1.18 (95% CI 1.01 – 1.37). No increased risk was found among women with a negative family history and non-proliferative findings.

Table 1: Breast cancer risk based on select clinical-epidemiologic factors

Characteristic	No. of Women	Person-Years	No. of Observed Events	No. of Expected Events	Relative Risk (95% CI)†
Overall	9087	144,881	707	453.0	1.56 (1.45–1.68)
Age at diagnosis of benign breast disease					
<30 yr	726	13,593	21	11.5	1.83 (1.13–2.80)
30–39 yr	1115	20,169	71	38.3	1.85 (1.45–2.34)
40–49 yr	2474	45,780	212	136.3	1.56 (1.35–1.78)
50–59 yr	2145	34,100	196	125.9	1.56 (1.35–1.79)
60–69 yr	1639	21,364	142	94.5	1.50 (1.27–1.77)
≥70 yr	988	9,874	65	46.6	1.40 (1.08–1.78)
Menopausal status‡					
Premenopausal (age <45 yr)	2948	54,419	169	106.1	1.59 (1.36–1.85)
Perimenopausal (age 45–55 yr)	2583	45,872	245	153.4	1.60 (1.40–1.81)
Postmenopausal (age >55 yr)	3556	44,590	293	193.6	1.51 (1.35–1.70)
Histologic findings					
Nonproliferative disease	6061	99,109	379	297.7	1.27 (1.15–1.41)
Proliferative disease without atypia	2690	41,610	264	140.2	1.88 (1.66–2.12)
Atypical hyperplasia	336	4,161	64	15.1	4.24 (3.26–5.41)
Family history of breast cancer§					
None	2668	44,974	171	145.4	1.18 (1.01–1.37)
Weak	1174	21,472	94	65.9	1.43 (1.15–1.75)
Strong	966	18,087	110	57.0	1.93 (1.58–2.32)

\* Numbers of women, person-years, and events may not sum to overall totals because of rounding.

† The relative risk reflects the observed number of events as compared with the number expected on the basis of Iowa SEER data. All analyses account for the effects of age and calendar period. CI denotes confidence interval.

‡ Menopausal status was categorized according to the age at breast biopsy.

§ Information on family history was available for 4808 of the 9087 women.

Hartmann et al., 2005, NEJM.

Over a 25 year period of time, the proportion of BBD with atypical hyperplasia in our cohort increased from 1.5% in the early part of the study to 5.6% in the latter part of the study (Table 2). Similarly, the proportion of proliferative disease without atypia also increased from 24.6% in the early 70s to 34.9% in the later years (see following table). The increased prevalence of proliferative disease, especially atypia, is attributed to the increased use of mammography leading to findings associated with microcalcifications.

Table 2: Histologies of BBD 1967 – 1991

		Year of Biopsy					All Subjects
		1967-71	1972-76	1977-81	1982-86	1987-91	
<b>Overall</b>		1038 (11.1%)	1876 (20.0%)	1546 (16.5%)	2334 (24.9%)	2582 (27.5%)	9376 (100%)
<b>Histology</b>	<b>NP</b>	754 (72.6%)	1379 (73.5%)	1086 (70%)	1490 (63.8%)	1535 (59.5%)	6244 (66.6%)
	<b>PDWA</b>	269 (25.9%)	462 (24.6%)	420 (27.2%)	748 (32.1%)	902 (34.9%)	2801 (29.9%)
	<b>AH</b>	15 (1.5%)	35 (1.9%)	40 (2.6%)	96 (4.1%)	145 (5.6%)	331 (3.5%)

NP=nonproliferative; PDWA=proliferative disease without atypia; AH=atypical hyperplasia.

**2. Atypia and risk.** We have studied our cohort of women with atypia (N=336) in depth. We have found that the risk of breast cancer was elevated for women with atypia and even greater for women with atypia who were under age 45 (RR=7.36). We examined risk by number of foci of atypia and found: 1 focus, RR=2.33; 2 foci, RR=5.41; and for three or more foci, RR=7.96 (see cumulative incidence figure below). Moreover, in the highest risk subgroup of women with three or more foci of atypia and histologic calcifications, the cumulative incidence exceeded 50% after 25 years. This level of risk approaches that reported for carriers of BRCA1/2 mutations. Risk was similar for ductal and lobular types of atypia; family history did not significantly increase risk. Breast cancer risk in women with atypia remained elevated over 20 years.

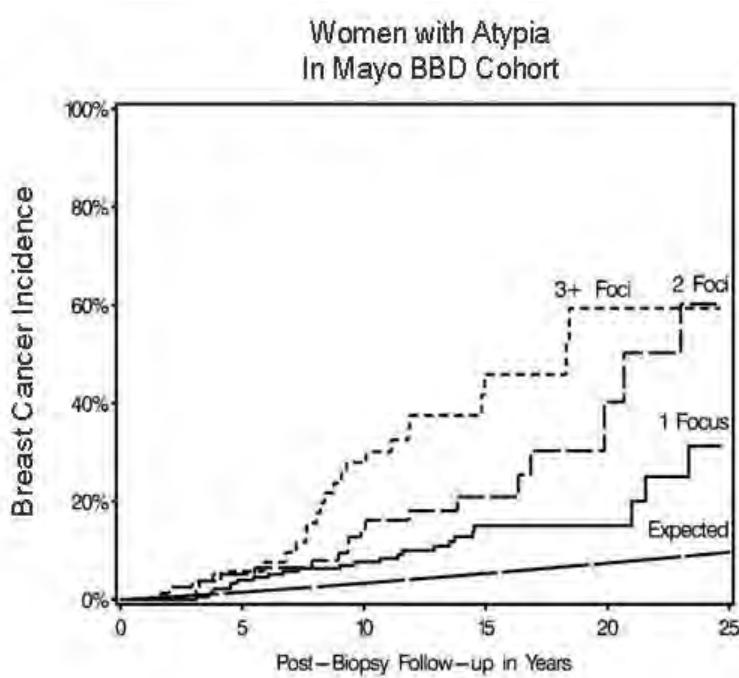


Figure 1: Observed cumulative breast cancer incidence among women with atypical hyperplasia, stratified by number of foci of atypia. Expected events calculated by applying age- and calendar period-stratified person years of observation among all women with atypia to corresponding Iowa SEER breast cancer incidence rates. Observed and expected events cumulated using Kaplan-Meier product limit methodology. (From Degnim AC, Visscher D, . . . Hartmann LC. Stratification of breast cancer risk in women with atypia: A Mayo cohort study, JCO, 2007).

**3. Papillomas.** The risk of breast cancer development in patients with papillomas, particularly those with multiple or atypical lesions, has been incompletely defined. We examined the association between breast papillomas and subsequent risk of breast cancer. We found that a single papilloma imparts a cancer risk similar to conventional proliferative fibrocystic disease. The presence of single papilloma with atypia does not modify the risk of atypical ductal hyperplasia/atypical lobular hyperplasia overall. The presence of multiple papillomas, however, increases the risk of breast cancer over that of proliferative fibrocystic disease (RR 3.01, 95% CI 1.10-6.55), even more so in women with multiple papillomas with atypia (RR 7.01, 95% CI 1.919-17.97). Thus, multiple papillomas constitute a proliferative breast disease subset having unique clinical and biologic behavior (Lewis JT et al. An analysis of breast cancer risk in women with single, multiple, and atypical papilloma, Am J Surg Pathol 2006;30:665-672).

**4. Radial Scars.** The significance of radial scars to subsequent risk of breast cancer has been debated. Radial scars (RS) are benign breast lesions of uncertain etiology. The growth pattern in RS can resemble breast cancer and on mammogram a RS can be difficult to distinguish from breast cancer, prompting biopsy. The literature is mixed about the risk of developing breast cancer following the diagnosis of RS, leading to our interest in examining the significance of RS in the subsequent development of breast cancer. We found no increased breast cancer risk for women with radial scars when compared to the risk already present due to proliferative disease with or without atypia. Breast cancer risk was not affected by the size or number of RS lesions (Berg JC et al. Breast cancer risk in women with radial scars in benign breast biopsies. Breast Cancer Res Treat 2008;108:167-174).

**5. Involution.** There are very few pathologic features identified thus far that are associated with a reduced risk of breast cancer. In our BBD resource, we studied if regression or involution of a woman's breast lobules (or terminal duct lobular units, TDLUs) was associated with later risk of breast cancer. The breast is organized into approximately 15-20 major lobes, each made up of lobules that contain the milk-forming acini. As a woman ages, these lobules are supposed to regress or involute with a reduction in the number and size of acini per lobule (see figure 2).

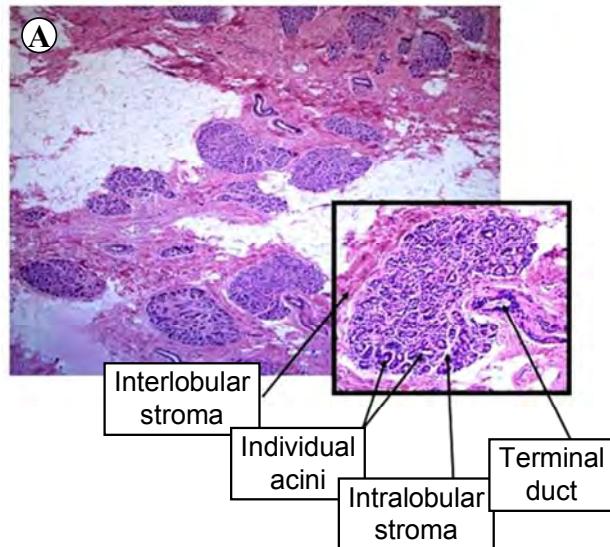
a. Qualitative Involution. Our study pathologist assessed the extent of involution in the background breast tissue of the women in our BBD cohort. Notably, those women who had complete involution of their TDLUs had a significantly lower risk of breast cancer (RR 0.91, 95% CI = 0.75 – 1.10) compared to those with partial (RR 1.47, 95% CI = 1.33 – 1.61) or no involution (RR = 1.88, 95% CI = 1.59 – 2.21) (Milanese TR et al. Age-related lobular involution and risk of breast cancer, JNCI 2006;98(22):1600-1607). We found that the presence of complete involution reduced risk even in women who were at high risk because they had atypia or a strong family history of breast cancer. This is a novel finding because the subject of age-related involution in relation to breast cancer risk has not been studied in the human. Importantly, this provides an additional feature to assess on a breast biopsy that allows us to fine-tune risk prediction for women. Secondly, and even more importantly, if the scientific community can determine what controls the

process of age-related breast involution, we may be able to induce it medically and thus, introduce a new “chemoprevention” strategy to offer women.

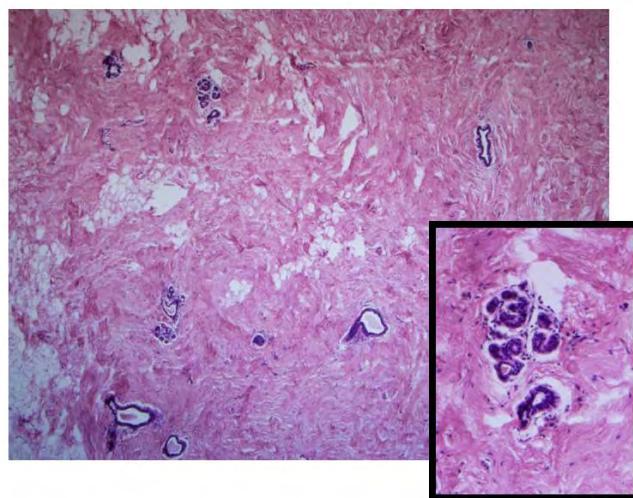
An editorial by Henson, Tarone and Nsouli accompanied our work in *JNCI* 2006. In this editorial they state “It is truly a remarkable event when traditional pathologic observations lead to new ideas about the prevention of cancer.....Results of the Mayo study provide a new paradigm for breast cancer research and prevention” (Henson DE, Tarone RE, Nsouli H. Lobular involution: the physiological prevention of breast cancer. 2006 *JNCI*;98(22):1589-90).

Figure 2. Histologic features of lobular involution. (a) A field of normal lobules or TDLUs, each comprised of multiple acini and specialized stroma (inset). (b) An example of complete lobular regression leaving small residual structures, largely depleted of acini (inset).

(a)



(b)



b. Quantitation of involution. We recently developed more quantitative measurements of lobular involution status to provide more objective assessment methods and to be able to more precisely identify the extent of involution (McKian KP et al. A novel breast tissue feature strongly associated with risk of breast cancer 2009 In press, JCO). To pursue this work, we identified 85 cases from our BBD cohort and matched 142 controls to them by age and year of BBD biopsy. One H & E stained slide per subject was scanned into the computer and analyzed using WebSlide Browser software (Bacus Labs). This software allows the measurement of structural features (lobular area, acini number) as visualized by light microscopy (Figure 3). The ten largest normal lobules were assessed for each patient by one observer without knowledge of case status or previous pathologic assessment (i.e. qualitative involution or histologic category). If fewer than ten normal lobules were present, all were assessed. Analysis included (i) counting the number of individual acini per lobular unit and (ii) delineating the circumference of the lobule to measure its area in square microns (Figure 3). We defined countable acini as nuclei forming a distinct circular pattern with or without the presence of a discernible lumen. Distinct lobules were defined by the presence of intersecting stromal tissue. Abnormal lobules, namely those that contained large portions of terminal ducts, atypical lobular or ductal hyperplasia, sclerosing adenosis, large cysts, or proliferative disease without atypia were not included in the analysis.

We found that this method for quantitating involution status was highly reproducible. Specifically, a random sample of 82 slides (25 cases and 57 controls) was read by a second observer using the quantitative, manual method described above. We compared the acinar count of the first and second readers, and there was strong correlation,  $r=0.91$  (5% CI: 0.87-0.94). We then compared acinar counts to other features, and found a strong association between involution extent judged qualitatively (none, partial, complete) and the acinar count and that involution extent was independent of histologic category (Figure 4A). Women with a positive family history were somewhat less likely to complete the process of involution (Figure 4A). We also found a strong correlation between breast cancer risk and acinar count (Figure 4B,C), as women who went on to develop breast cancer had significantly more acini/lobule (less involution).

We compared the acinar count of cases vs controls. Women who developed BC had significantly more acini per lobule (24.3) than women who remained unaffected (17.8) ( $p=0.0008$ ). Dividing acinar count into categories of ten, we observed a step-wise increase in risk of BC with increasing numbers of acini/lobule ( $p=0.0004$ ), as shown in Figure 4B. We also plotted BC risk by acinar count, demonstrating the continuous nature of this risk feature (see spline in Figure 4C).

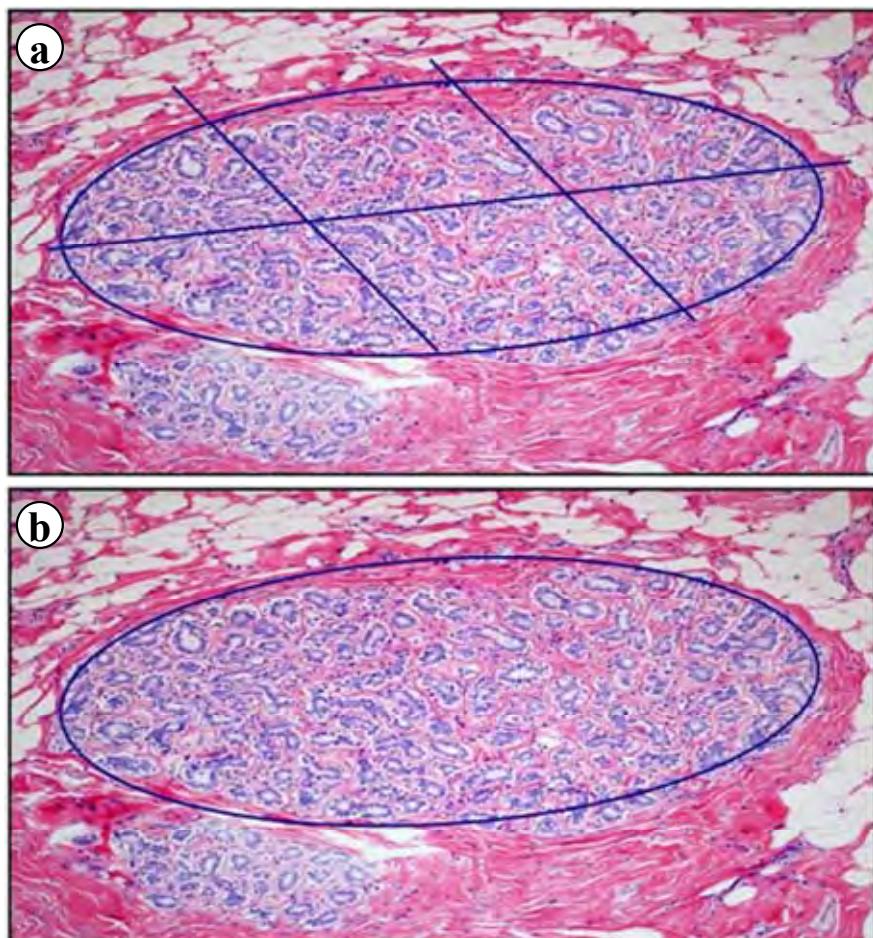


Figure2. Quantitative LI. **a**, Quantification by number of acini per lobule. **b**, Quantification by lobular area in  $\mu^2$ .

**A**

Extent of Involution (Qualitative) (p<0.0001)	Acinar Count
None	32.0 (26.4-38.8)
Partial	19.7 (17.5-22.2)
Complete	7.7 (5.8-10.3)
<b>Histologic Category (p=0.31)</b>	
Non-proliferative	18.8 (16.1-22.0)
Proliferative disease without atypia	22.1 (18.6-26.3)
Atypical hyperplasia	18.6 (13.2-26.2)
<b>Family History (p=0.07)</b>	
Negative	18.3 (16.4-21.6)
Positive	22.9 (19.0-27.6)
<b>Breast Cancer Status (p=0.0008)</b>	
Case	24.3 (20.6-28.6)
Control	17.8 (15.4-20.4)

**B**

Association of Acinar Count and BC Risk

Number of acini/lobule	Number of women	Lobule RR (95% CI)	P-value
0-10	53	1.00 (ref)	0.0004
11-20	63	2.80 (0.88-8.93)	
21-30	39	3.23 (0.87-11.94)	
31-40	26	3.23 (0.72-14.41)	
41 or more	46	11.85 (2.92-48.12)	

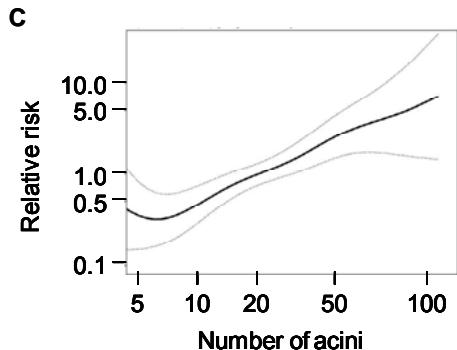


Figure 4. Quantitative LI and cancer risk. (A) Correlations between acinar count, other variables, and case status. (B) Risk associated with quantitative LI as a discrete variable. (C) Risk associated with quantitative LI as a continuous variable.

c. Concordance of involution measures across breast tissue. We found that measurements of lobular involution are highly consistent across multiple areas of a woman's breast (Vierkant RA et al. Lobular involution: localized phenomenon or field effect? 2008 Breast Cancer Res Treat, epub ahead of print). For this study, the tissue from 15 women who had undergone bilateral prophylactic mastectomy was analyzed. Specifically, we chose a single section of breast tissue from each quadrant of both breasts. An H&E stained section was prepared from each specimen, and the extent of involution was

categorized as none (0%), mild (1-24%), moderate (25-74%) or complete ( $\geq 75\%$  involuted lobules). Within-woman concordance of involution was calculated using intraclass correlation coefficients (ICCs) which indicated strong correlation among involution measures (Table 3). Correlations were also similar when modeling each breast as an experimental unit, which confirmed that patterns of involution were uniform in the entire field of breast tissue.

Measure	Result
Treating each woman as the experimental unit	
Intraclass correlation (95% CI)	0.75 (0.59, 0.89)
Kappa coefficient (95% CI)	0.67 (0.59, 0.75)
Pairwise comparisons, $N$ (%) <sup>a</sup>	
Perfect matches	341 (81)
Partial matches	76 (18)
Non-matches	3 (1)
Treating each breast as the experimental unit	
Intraclass correlation (95% CI)	0.74 (0.60, 0.85)
Kappa coefficient (95% CI)	0.66 (0.53, 0.78)
Pairwise comparisons, $N$ (%) <sup>a</sup>	
Perfect matches	145 (81)
Partial matches	34 (19)
Non-matches	1 (1)

Table 3. Measures of concordance of involution in 15 women undergoing prophylactic mastectomy. Eight quadrants assessed per woman. Data from Vierkant RA et al. 2008.

### ***C. Perform centromere studies***

Most invasive breast cancers, like many other solid tumors, have amplified centrosomes. The extent of centrosome amplification correlates with the levels of chromosomal instability in invasive ductal carcinoma of the breast. Centrosome amplification is also present in ductal carcinoma in situ, but has not been investigated in benign breast lesions. In our pilot study, we investigated the status of centrosomes in benign breast lesions of

various histologies to determine if amplified centrosomes can be detected in the absence of malignancy and invasion, and if any histologic types of benign breast lesions have significant levels of centrosome amplification.

We selected paraffin-embedded tissue blocks from women with non-proliferative lesions, proliferative lesions without atypia, and atypical hyperplasia. We had previously determined the relative risks of developing breast cancer associated with these lesions in our large cohort of women; the relative risk associated with non-proliferative lesions was 1.27 (95% CI 1.15-1.41), 1.88 (95% CI 1.66-2.12) in proliferative lesions without atypia, and 4.24 (95% CI 3.26-5.41) in lesions with atypia. Serial sections were cut to allow for staining with hematoxylin and eosin, gamma tubulin, and cyclin D1 on adjacent slides. The lesions of interest were circled by the study pathologist (DV) on the H&E or cyclin D1 stained slides. These slides were then scanned using a digital imaging system. The corresponding area was marked on the immunofluorescent slide stained with gamma tubulin antibodies to facilitate locating the lesion at high magnification.

Centrosome amplification was seen infrequently in non-proliferative lesions and in proliferative lesions without atypia. However, about 88% of atypical hyperplasia lesions had detectable centrosome amplification and about 30% had moderate to considerable levels of centrosome amplification (see Figures 5 and 6 below). Thus, centrosome amplification is seen more frequently in benign lesions having the highest relative risk of developing breast cancer.

This is the first quantitative demonstration of centrosome amplification in benign lesions. These pilot data demonstrate that centrosome amplification is more prevalent in atypical hyperplasia lesions, and these lesions are associated with the highest relative risk of developing breast cancer (Lingle W et al. Centrosome amplification is greatest in benign breast lesions associated with an increase in risk of cancer. San Antonio Breast Cancer Symposium, annual meeting, 2006, San Antonio, TX).

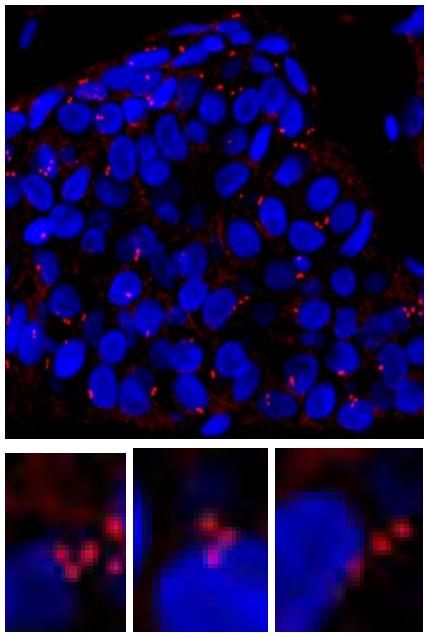


Figure 5. Immunofluorescence staining for centrosomes (red) in an atypical hyperplasia. Many nuclei (blue) have more than 2 centrosomes associated with them. Normal cells have 1 centrosome during G1 of the cell cycle and 2 centrosomes during G2 of the cell cycle.

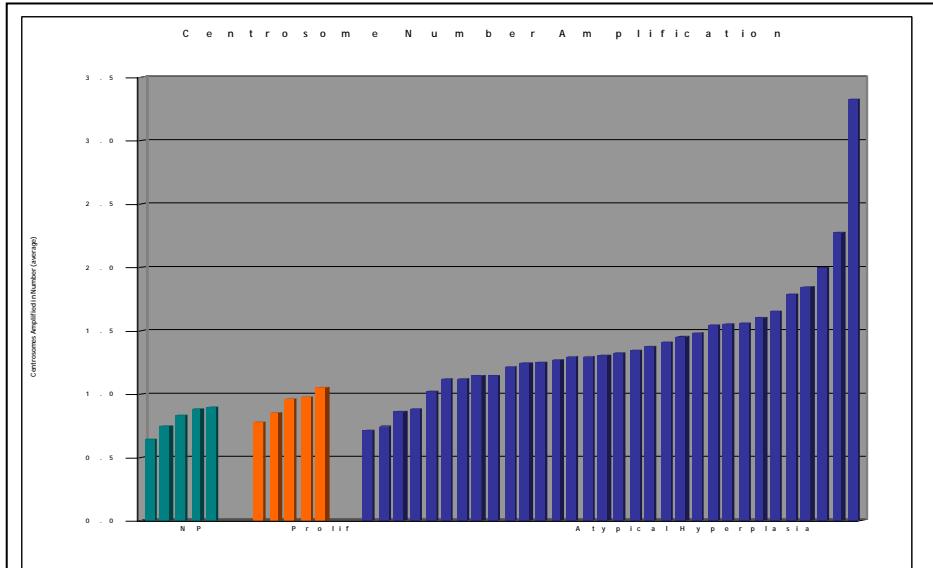


Figure 6. Each bar represents the average centrosome number in an individual lesion. More than one centrosome per cell on average is found in 88% of atypical hyperplasia samples, compared to only 9% of the other BBD types. The range is also greater in atypical hyperplasias.

#### D. Individual biomarkers

**1. COX-2.** COX-2 is a very important mediator of biologic processes during inflammation and cancer. Through work of our UCSF study team led by Dr. Thea Tlsty and other labs, as described in Section III, we know that COX-2 expression is up-regulated in invasive breast cancer and also in ductal carcinoma in situ. We sought to determine if increased expression occurred a step earlier — namely in women with atypia — and if the presence of high levels of COX-2 would predict which women with atypia would go on to develop breast cancer. In fact, we found that moderate to strong COX-2 expression is associated with a significantly greater likelihood of a subsequent breast cancer in women with atypia. (See figure below). For women whose atypia lesion exhibited negligible (0-1+) staining, their likelihood of developing breast cancer was 13% at 15 years from biopsy, vs. 25% for those with 3+ COX-2 staining (Visscher DW et al. Association between cyclooxygenase-2 expression in atypical hyperplasia and risk of breast cancer. JNCI. 2008;100(6):421-7).

Besides its potential for risk prediction, COX-2 represents a molecular target for chemoprevention strategies. COX-2 inhibitors are available pharmaceutically and in fact,

epidemiologic studies have shown that women who have taken COX-2 inhibitors for arthritis have a lower chance of developing breast cancer.

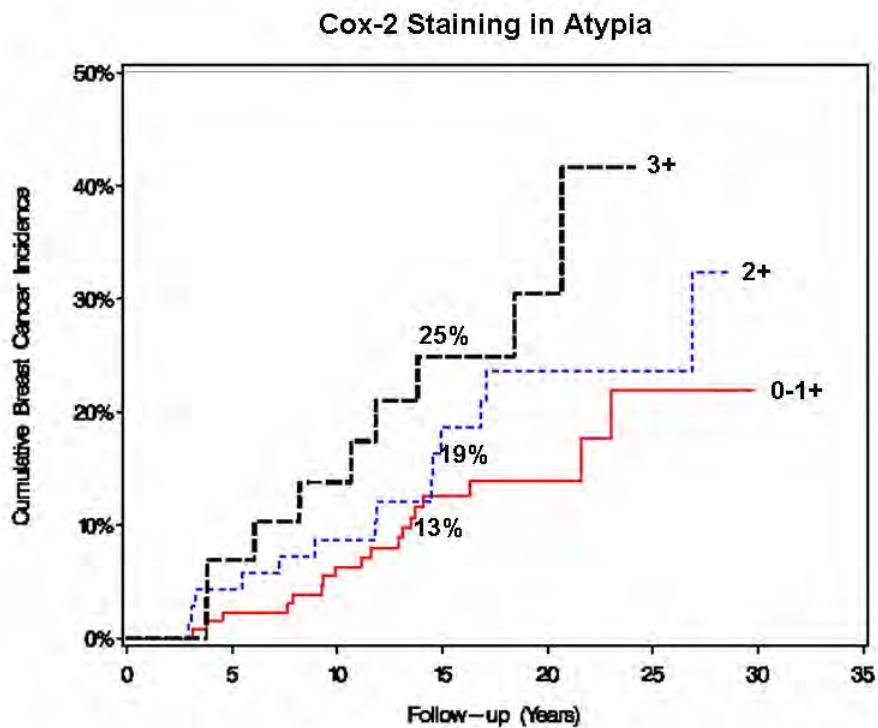


Figure 7. COX-2 staining in atypia. Visscher DW et al. Association between cyclooxygenase-2 expression in atypical hyperplasia and risk of breast cancer. JNCI 2008;100(6):421-7.

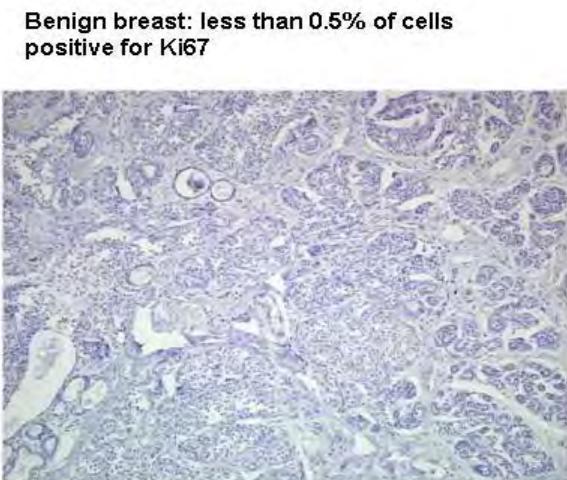
**2. ER.** The estrogen receptor is essential to mediate the growth regulatory signals of estrogen in normal breast tissue and serves as a therapeutic target and predictive factor in breast cancer. The extent of ER staining in a well-characterized cohort of women with atypia, to our knowledge, has not yet been reported.

We used the Automated Cellular Imaging System III (ACIS) to evaluate the intensity and percent ER staining in 231 women with atypia. This system is able to provide automated quantification of biomarkers. The areas of atypia were identified by the study pathologist and read into ACIS with reports produced for each identified area of interest. The preliminary analysis of the 10 most intensely stained areas showed a mean of 56 percent stained cells (standard deviation 30.78, range 0.00 – 99.99). The mean intensity was 114 (standard deviation 28.97, range 0-206 (possible range 0 -256)). A linear multivariate mixed model examined percent staining and intensity differences based on atypia type (lobular, ductal), cancer status, and year of biopsy after controlling for repeated measures within a woman.

This initial analysis showed a stronger staining intensity and a greater percentage of staining of atypical ductal hyperplasia compared to atypical lobular hyperplasia (intensity: ADH mean of 117.68, standard error 2.5224 and ALH mean of 100.32, standard error 2.4537,  $p < 0.0001$ ; percentage: ADH mean of 64.1887, standard error 2.7860 and ALH mean of 44.2121, standard error 2.6720,  $p < 0.0001$ ) (Barr-Fritcher EG et al. Estrogen receptor expression in atypical hyperplasia and its association with type of atypia and age. 2009; United States and Canadian Academy of Pathology annual meeting). We have completed the ER assessments for all areas of atypia and are currently completing our analysis of these data.

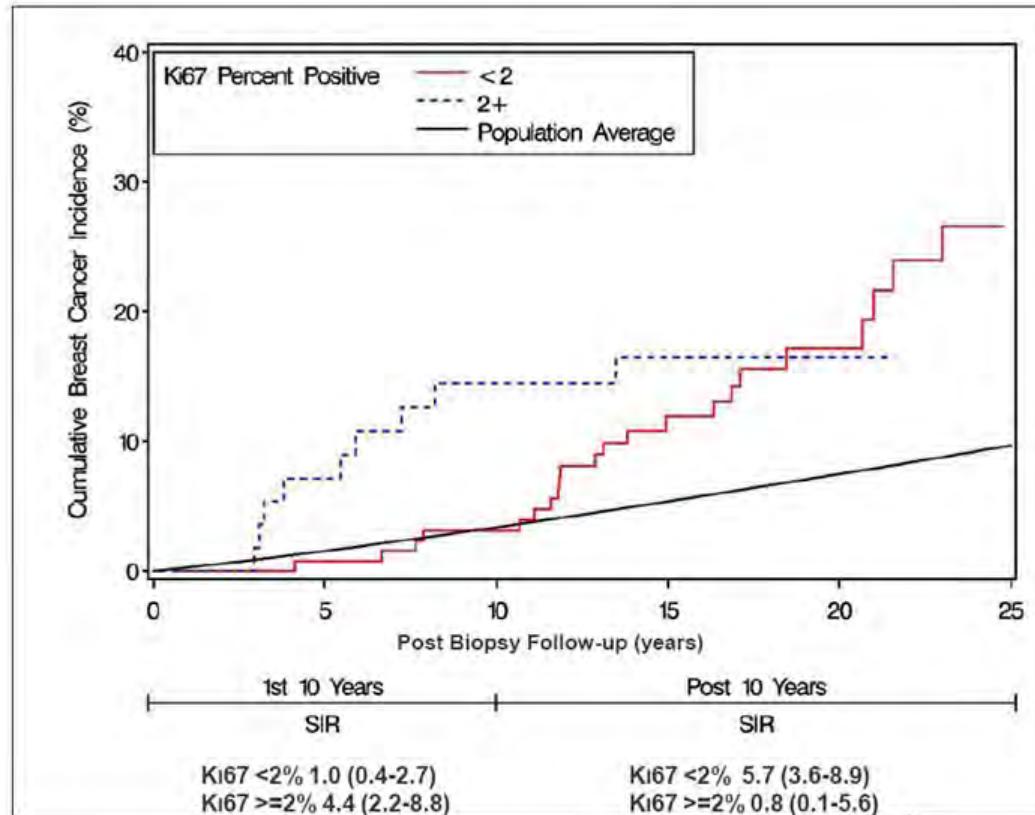
**3. Ki67.** Multiple studies have shown that breast cancers with higher proliferation rates are associated with worse outcome. Ki67 is the best characterized proliferation marker. To our knowledge, there has not been a study of Ki67 in the setting of BBD — to check for association with the later development of breast cancer.

Figure 8: Ki67 staining



Here we show the results in a group of 192 women with BBD with all samples stained for Ki67. 32 women have developed breast cancer.

Figure 9: Ki67 stain percentage and breast cancer incidence



One of the strengths of our study design, namely that of a retrospective cohort, is that we have long follow up and can thus evaluate potential time-varying risk factors such as proliferation. Those women whose atypias had a higher proliferative component (dotted line) were more likely to develop breast cancer within the first 10 years following their biopsy. Those with fewer proliferating cells were still at increased risk for breast cancer, but their cancers occurred later, after 10 years following the biopsy.

The clinical value of this feature is as follows: if we focus on the first 10 years after a biopsy of atypia, we see that there is a distinct difference in risk between those women with high vs. low proliferation in their sample. If there is low proliferation, the woman's risk is not increased above that of normal age matched women within the first 10 years. This is exactly the type of information we need to stratify women into different risk groups over different time intervals.

### III. Discovery – *In Vitro* Culturings and Gene Profiling Studies

#### A. Culture BBD specimens and document their growth characteristics

Preliminary growth curves and characteristic micrographs on 14 samples are shown in the Figure below. Of these 14 cell culture samples, 8 were generated from breast tissue of pre-menopausal women and the remaining 5 from post-menopausal breast tissue. UCSF extracted RNA from these samples and reverse transcribed the first and second strand cDNA using Ambion Message Amp kit.

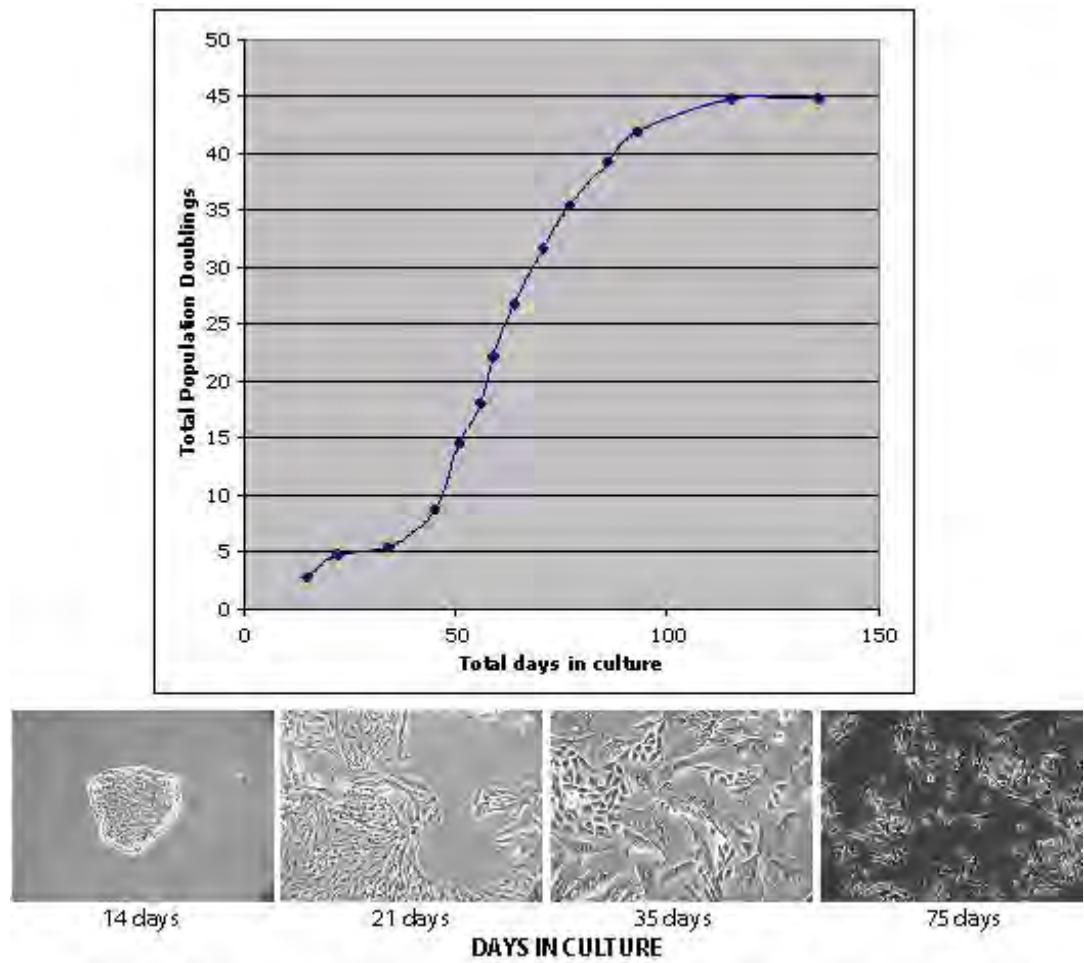


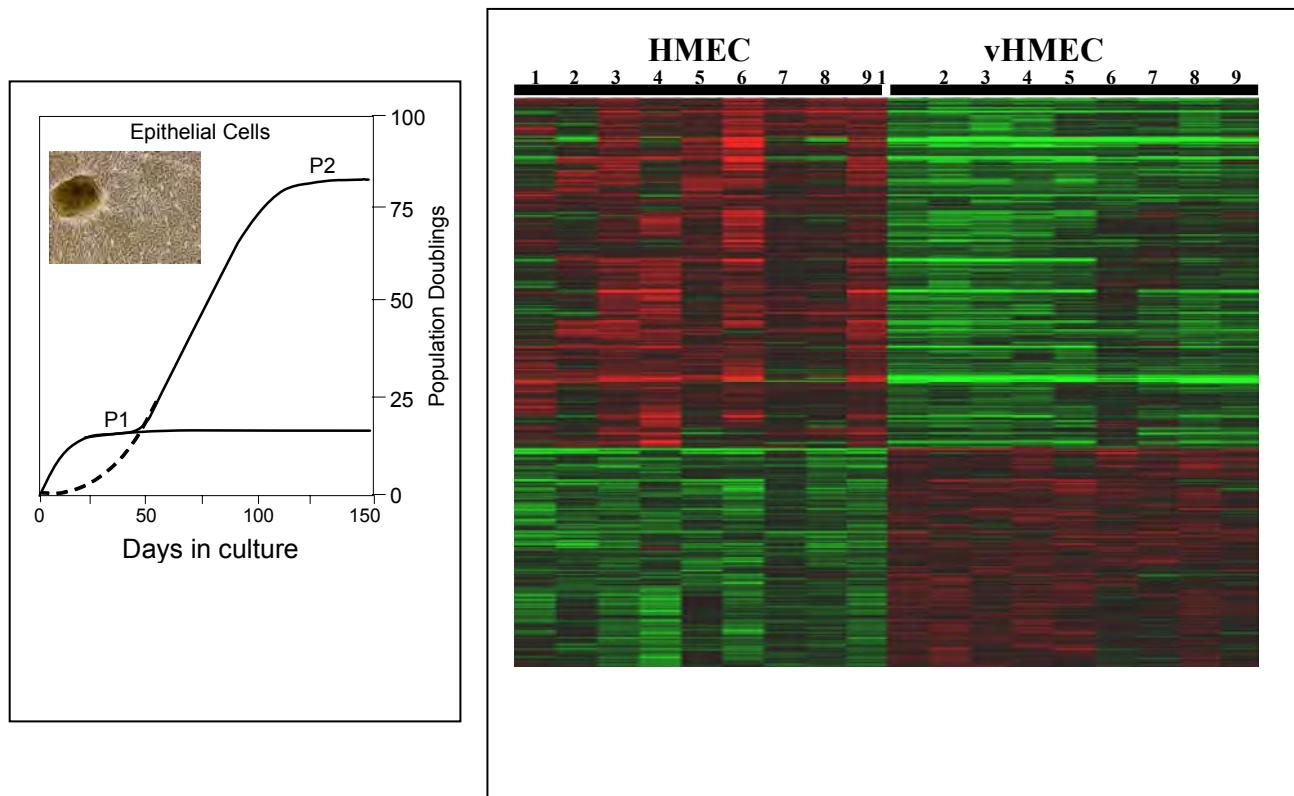
Figure 10. Representative growth curve and micrographs (4X) of BBD samples grown in culture.

#### B. Profile BBD specimens

Epithelial cells were isolated and propagated from disease-free breast tissue and tissue containing BBD to determine the growth kinetics of BBD epithelial cells. All BBD tissue generated two epithelial populations with distinct growth characteristics, similar to epithelial cells generated from disease-free breast tissue. Briefly, the first population of human mammary epithelial cells (HMEC) grows in culture for approximately 10-15 population doublings before reaching an irreversible p16-dependent growth arrest termed P1. The second population, variant HMEC (vHMEC), grew in culture for an additional 40-50 population doublings due to the loss of p16/Rb signaling before reaching a telomere-dependent growth arrest. We hypothesized that the identification of molecular

alterations that accompany the extended proliferative capacity of the vHMEC population prior to telomere attrition and genomic instability may provide potential relevant biomarkers of risk. To this end we analyzed the global transcript levels of nine isogenic HMEC and vHMEC populations. Unsupervised hierarchical clustering analysis identified approximately 1240 genes that significantly differentiated the two populations on the basis of expression patterns. We found that many differentially expressed genes in vHMEC resembled expression of genes in DCIS and invasive cancer. These data support the utility of this model for the discovery of novel biomarkers for risk assessment. We chose a subset of 512 genes that robustly stratified the two groups (figure below). Many of the differentially expressed genes in the variant population are known E2F downstream targets, such as survivin, forkhead D1, BUB1 and Rad51. However, many have no known association with p16/Rb signaling, suggesting that the vHMEC are a unique population of cells.

Figure 11. Profile BBD specimens



**1. COX-2.** COX-2 was identified as one of the most robustly upregulated genes in vHMEC. The sustained expression of COX-2 in the vHMEC population was an intriguing finding because COX-2 is a stress activated gene that is tightly regulated in normal cells, such that it is only transiently expressed in response to cellular stress. This finding in the vHMEC cells suggests that this subpopulation exhibits a sustained stress response compared to the majority of epithelial cells. We find that HMEC (normal primary cells) are refractory to COX-2 induction in response to exogenous stress induced by inflammatory cytokines, DNA or microtubule damage, and oncogene-induced stress (adjacent figure). This differential induction of COX-2 may reflect a potential for transformation since COX-2

overexpression is accompanied by phenotypes that are critically relevant to cancer development, such as promoting proliferation, invasion and angiogenesis as well as inhibiting apoptosis and immune surveillance. This hypothesis is supported by our observations that forced expression of COX-2 in HMEC by retroviral infection produced enlarged flattened cells that were growth arrested (adjacent figure). Cell morphology and proliferation was not altered in vHMEC

constitutively expressing COX-2. We find that the molecular changes underlying the differential phenotypic response to COX-2 overexpression are dependent on p16/Rb signaling. HMEC overexpressing COX-2 resulted in elevated p16, p53 and p21 and downregulation of Rb (see figure). This is in contrast to p16 silenced vHMEC where overexpression of COX-2 did not alter the level of p53 or p21. Thus, in normal cells, COX-2 induces a cell cycle arrest through the upregulation of p16 and p53 to protect cells from inappropriate oncogenic signaling. In cells that have lost p16/Rb signaling, COX-2 overexpression does not induce a growth arrest. We argue that sustained stress activation in the absence of growth arrest defines an aberrant stress phenotype that may set the stage for carcinogenesis.

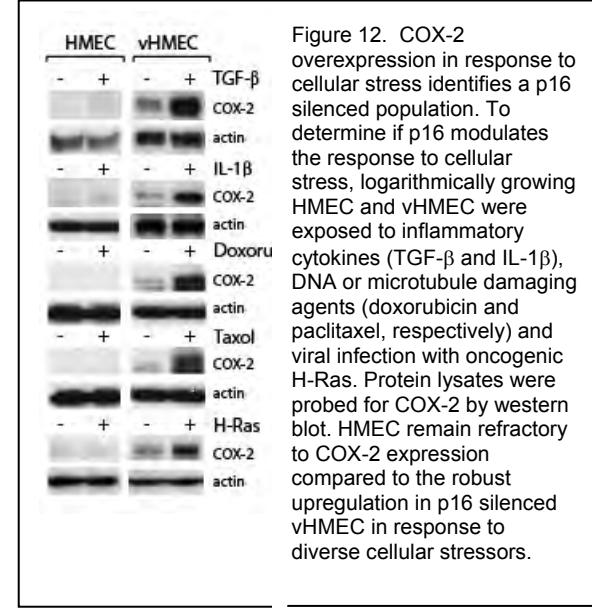


Figure 12. COX-2 overexpression in response to cellular stress identifies a p16 silenced population. To determine if p16 modulates the response to cellular stress, logarithmically growing HMEC and vHMEC were exposed to inflammatory cytokines (TGF- $\beta$  and IL-1 $\beta$ ), DNA or microtubule damaging agents (doxorubicin and paclitaxel, respectively) and viral infection with oncogenic H-Ras. Protein lysates were probed for COX-2 by western blot. HMEC remain refractory to COX-2 expression compared to the robust upregulation in p16 silenced vHMEC in response to diverse cellular stressors.

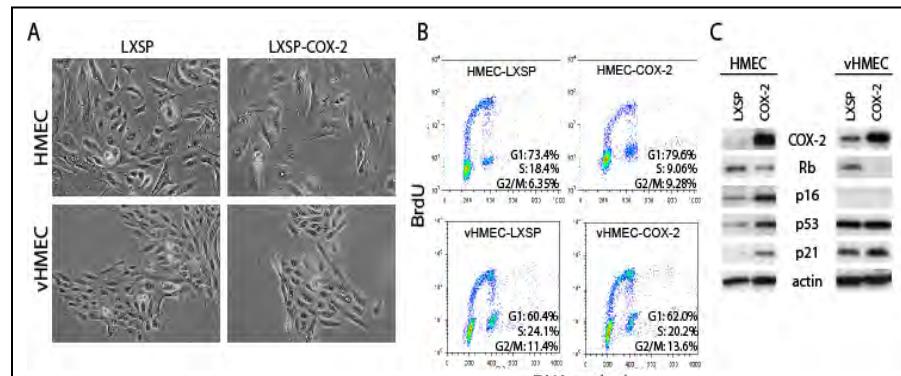


Figure 13. COX-2 overexpression differentially induces cell arrest in HMEC versus vHMEC. To determine if COX-2 overexpression differentially modulates proliferation in p16 silenced cells, HMEC and vHMEC were infected with retrovirus containing an empty vector LXSP or LXSP-COX-2. Cells collected 4-6 days post infection were visualized by phase contrast microscopy (A) pulsed with BrdU for cell cycle analysis (B). COX-2 overexpression was confirmed by western blot (A). Overexpression of COX-2 in HMEC causes the upregulation of p16, p53 and p21 (A) leading to cell cycle arrest (B). In contrast, COX-2 constitutive expression in vHMEC did not alter protein levels of p53 or p21, nor was the cell cycle distribution significantly altered.

**2. p16.** Our in vitro model demonstrated an inverse relationship between p16 and COX-2 expression, as shown in this figure. This finding prompted us to determine if loss of p16/Rb was sufficient to induce COX-2 expression. We found that sequence specific silencing of p16 causes COX-2 upregulation and provides cells with a proliferative advantage. Although genetic downregulation of p16 did not result in robust COX-2 upregulation, cells became responsive to exogenous induction of COX-2 by TGF- $\beta$ , as shown. Since p16 exerts many of its biological effects through Rb, we determined if induction of COX-2 is mediated through Rb. We found that downregulation of Rb by retroviral infection of HMEC with the human papilloma virus E7 (HPV-E7) caused a robust upregulation of COX-2 expression and sensitizes cells to COX-2 induction by exogenous inducers such as TGF- $\beta$ . The absence of Rb also provided a proliferative advantage. Thus, loss of p16 or Rb causes the upregulation of COX-2 and provides cells with a proliferative advantage, thereby mimicking the aberrant stress phenotype described previously in the vHMEC cells. We next sought to determine if p16/Rb signaling is clinically significant.

The majority of normal breast tissue is devoid of p16 immunostaining. Specifically, we observed that only 10% of disease-free tissue contains >30% of the lobules positive for p16. This is in contrast to either pre-malignant or malignant breast lesions. Twenty seven percent of ADH lesions display heterogeneous immunostaining for p16, a significant upregulation ( $P=0.05$ ) compared to normal tissue. This level of immunopositivity and heterogeneity is maintained in low, intermediate and high grade DCIS lesions. The level of p16 positivity in invasive tumors is similar to that observed in DCIS. However, in contrast to DCIS, the pattern of p16 staining in invasive tumors is much more homogeneous.

In normal cells overexpression of p16 causes a cell arrest that acts as a protective mechanism in response to diverse cellular stressors or inappropriate mitogenic stimulation. To determine if the upregulation of p16 we observe during pre-malignancy

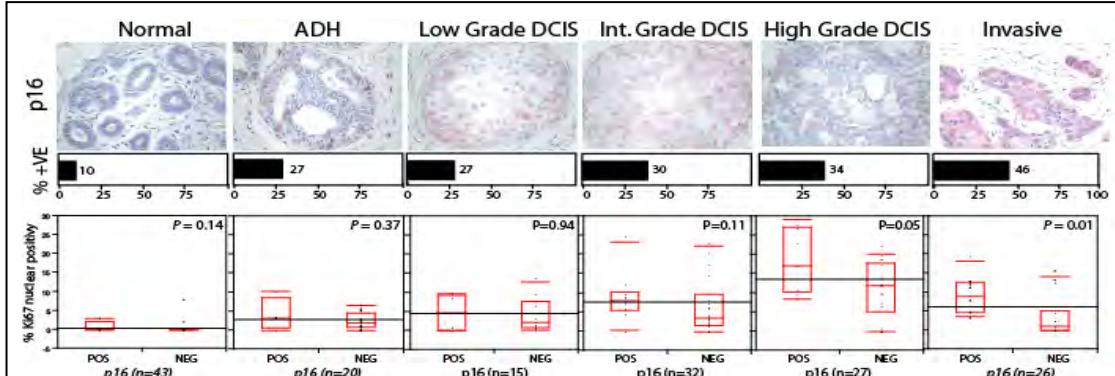


Figure 14. Loss of p16/Rb signaling correlates with proliferation in high grade DCIS and invasive carcinoma. Representative p16 immunostaining in normal breast tissue, atypical ductal hyperplasia (ADH), low, intermediate and high grade ductal carcinoma in situ (DCIS) and invasive carcinoma are illustrated. The bar graph demonstrates the percent positive cases. Box plots represent the correlation between p16 immunopositivity or negativity with Ki67 for each tissue type. Note that p16 overexpression correlates with Ki67 in high grade DCIS and invasive carcinoma. .

is accompanied by cell arrest, we determined the relationship between p16 and

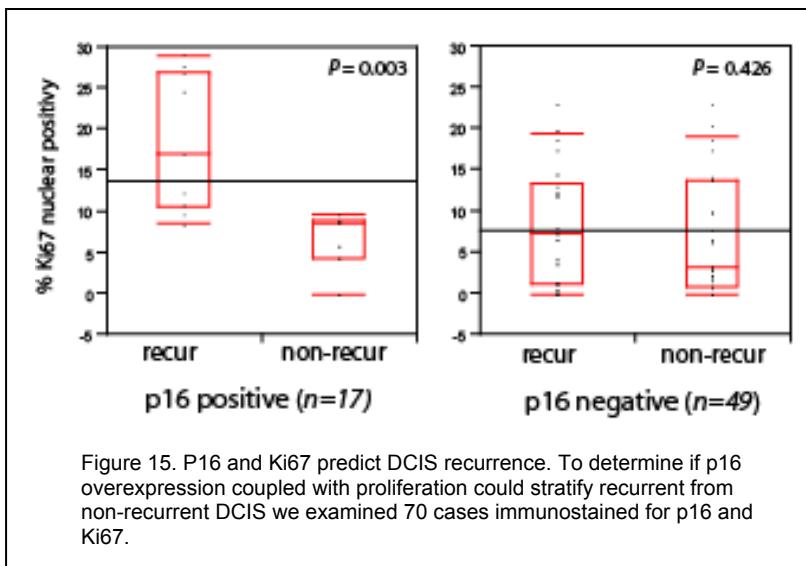


Figure 15. P16 and Ki67 predict DCIS recurrence. To determine if p16 overexpression coupled with proliferation could stratify recurrent from non-recurrent DCIS we examined 70 cases immunostained for p16 and Ki67.

correlated with elevated Ki67 index labeling. The finding that p16 and Ki67 are linked in high grade DCIS and invasive breast tumors strongly suggests that p16-mediated regulation of cell cycle is abrogated. Therefore, p16 overexpression in high grade DCIS and invasive tumors is dysfunctional.

We reasoned that loss of p16/Rb signaling may cause cells to become refractory to stress-induced growth arrest and may reflect a more aggressive phenotype. To determine if p16 overexpression and Ki67 index labeling could stratify recurrent from non-recurrent DCIS we examined a series of 70 DCIS cases with known outcome. We find that coupling p16 and Ki67 indeed identifies DCIS cases that recur (see figure 15).

To further pursue these observations, we have stained our atypia samples for p16 by immunohistochemistry. These samples have been read by the Tlsty team at UCSF and final analyses are currently underway.

### C. Profiling of involuted vs. noninvoluted samples

To assess the feasibility of defining biomarkers from paraffin-embedded samples in our BBD cohort, we obtained RNA from patients identified as noninvoluted (N=8) and involuted (N=6), matched for age of patient and year of biopsy. We performed profiling analyses using DASL whole genome microarray chips. Twelve of these fourteen samples (86%) produced excellent expression results (for each of these twelve, more than 65% of transcripts had a detection-p-value less than 0.05, indicating these transcripts were detected). The remaining two samples produced expression results too poor to be analyzed (for each of these two, more than 50% of transcripts had a detection p-value greater than 0.05 indicating they were not detected). A heatmap of the top 450 differentially expressed gene expression profiles shows both up and down regulated genes which may be useful for segregating the involuted from noninvoluted samples (Figure 16a). We converted the RNA to cDNA and assessed expression levels by quantitative PCR of six transcripts that were significantly differentially expressed by the microarray analysis between involuted and noninvoluted samples. We found that three of these showed

proliferation in archival tissue samples (see figure). The lesions that stained positive for p16 in normal breast tissue, ADH, low and intermediate grade DCIS showed no relationship with Ki67. These data suggest that p16 may be functionally exerting a cell cycle arrest in these tissues. In contrast, high grade DCIS lesions and invasive tumors overexpressing p16 were

statistically significant differences ( $p < 0.05$ ) between the two sample groups via PCR (Figure 16b): FANCD2, a homologue of FANCD1/BRCA2 that has been associated with increased risk of sporadic breast cancer (Barroso E, Milne RL, Fernandez LP, Zamora P, Arias JI, Benitez J, Ribas G: FANCD2 associated with sporadic breast cancer risk. *Carcinogenesis* 2006; 27(9):1930-7), CD34, a marker of hematopoietic stem/progenitor cells, fibroblasts, and vascular cells that shows decreased expression in breast cancer, and WNT10A, a marker previously implicated in embryonic developmental pathways that shows decreased expression in breast cancer (Kirikoshi H, Inoue S, Sekihara H, Katoh M: Expression of WNT10A in human cancer. *Int J Oncol* 2001; 19(5):997-1001). We evaluated expression of these proteins by IHC and found results consistent with those obtained by quantitative RT-PCR (Figure 16c). Thus, we show that we can successfully identify differentially expressed genes to evaluate as potential biomarkers of involution from FFPE biopsies.

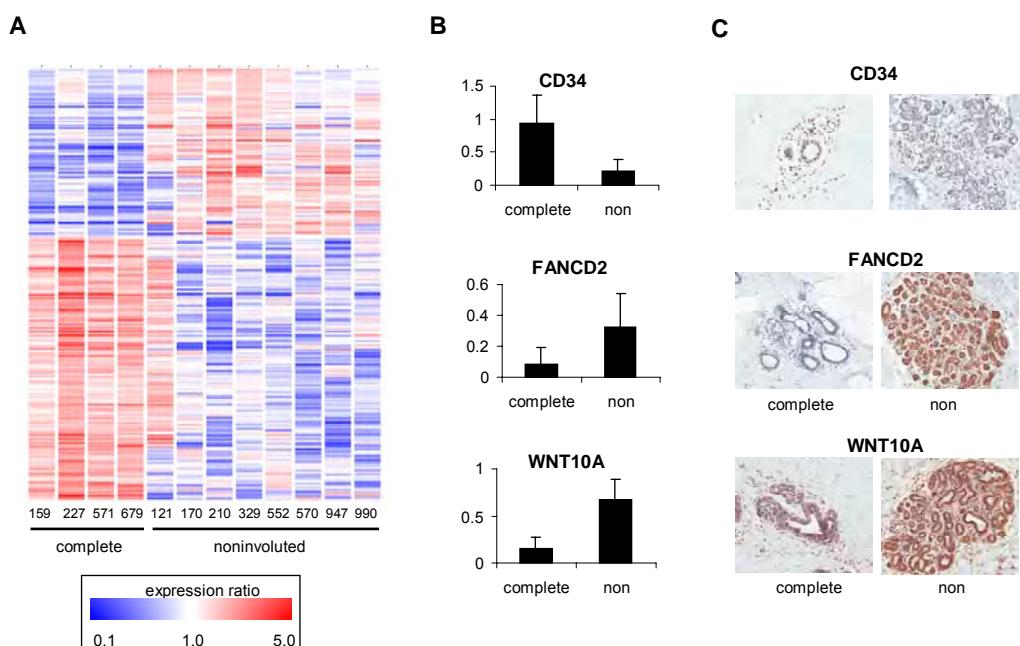


Figure 16. (A) Gene expression fingerprint of RNA derived from FFPE samples of patients identified as showing complete involution (complete,  $N=4$ ) or no involution (noninvoluted,  $N=8$ ). (B) Quantitative PCR analysis of three transcripts predicted to distinguish complete involuted vs noninvoluted patient samples. (C) Immunohistochemistry analysis of the three biomarkers in complete involuted and noninvoluted biopsies. *Unpublished data.*

#### IV. Compare BBD in African-American vs. Caucasian-American women

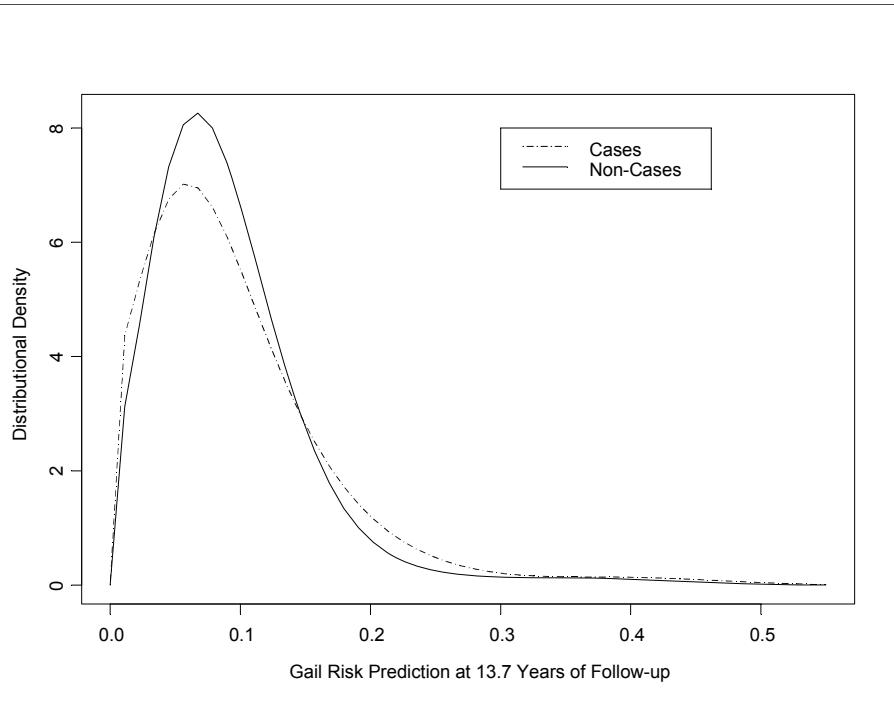
A better understanding of breast cancer risk and precursor lesions is particularly important in the African-American population as these women are diagnosed at a younger age and tend to have higher grade and more advanced stages than Caucasian-American women. African-American women also experience a significantly higher mortality from breast cancer than Caucasian-American women. Dr. Hind Nassar has read the histology on African-American women with BBD diagnosed at Wayne State University/Karmanos Cancer Institute between 1/1/1998

and 12/31/2000. All women were between 18 and 85. Exclusions are the same as those for the Mayo cohort and include a history of invasive or in situ breast carcinoma prior to, or within six months of the benign breast biopsy, unilateral or bilateral mastectomy prior or at diagnosis, breast reduction and the following histologies: lipoma, fat necrosis, epidermal cysts, hematoma, accessory structure, phyllodes tumor, lymph node with no breast tissue. The resulting sample included 1,126 African-American women, with a mean age at time of biopsy of 47.8 years (SD = 13.92). Breast cancer has been identified thus far in 32 women (2.8%) at a mean follow-up of 8.9 years (SD = 2.13). Our findings thus far are that African-American women are younger at diagnosis and have more nonproliferative and mass forming lesions than Caucasian-American women. Additionally, lobular involution appears to occur at a slower rate in African-American women than Caucasian-American women.

## **V. Assess accuracy of Gail model.**

The Gail model is currently the main tool used in the clinical setting for risk assessment in patients with atypia. This occurs despite the fact that the Gail model has not been validated in this group of patients. Thus, we evaluated the Gail model in our group of women with atypia. We used this model to predict 5 year and follow-up specific risks for each woman and compared the Gail model predictions to actual observed breast cancers in the group. Considering all women with atypia, the Gail model over-estimated the number of breast cancers that would occur in the first 5 years after biopsy (12.8 predicted, 8 occurred). However, when using all years of available follow-up for the group, the Gail model underestimated the risk of breast cancer in women with atypia (Gail model predicted 31.7 breast cancers while 58 occurred). Additionally, we found the concordance between Gail model individual-specific predicted outcomes and observed outcomes to be 0.50 (95% CI 0.44 – 0.55), no better than chance alone. As shown in the figure below, the Gail model predictions for the 58 women with atypia who developed breast cancer (cases) were superimposed on those of the 273 women who did not develop breast cancer (noncases). This has obvious implications for clinical practice. Healthcare providers should be cautious when using the Gail model in counseling patients with atypia regarding their risk of developing breast cancer.

Figure 17. Distributions of Gail model risk probabilities. Plot contains estimates for individualized risk at the end of the available follow-up. As risk predictions depend on age at BBD, and length of follow-up, the risk predictions were corrected for these factors prior to comparison.



## VI. Establish a relational database

We created a Sybase database to track tissue samples(paraffin embedded blocks, tissue slides and pieces of tissue) as they are moved between laboratory locations and to manage the biomarker result data. A web-based interface to this database has been created. All samples (individually or in boxes) are tracked using bar codes. The interface allows users to scan the bar code of the sample labels and enter information as they perform a task such as moving a sample, creating a slide, or entering laboratory results.

The process begins as the information on the paraffin blocks is stored in the database. These blocks are inserted into barcode labeled boxes which are scanned whenever they are moved to a new location. In a processing laboratory, the blocks are cut and the tissue slices are affixed to barcode labeled slides or – for very thick slices – placed into vials. The slides are scanned as they are put into slide boxes. The boxes in turn are scanned as they move to new locations and when identical laboratory procedures (e.g., a tissue stain)

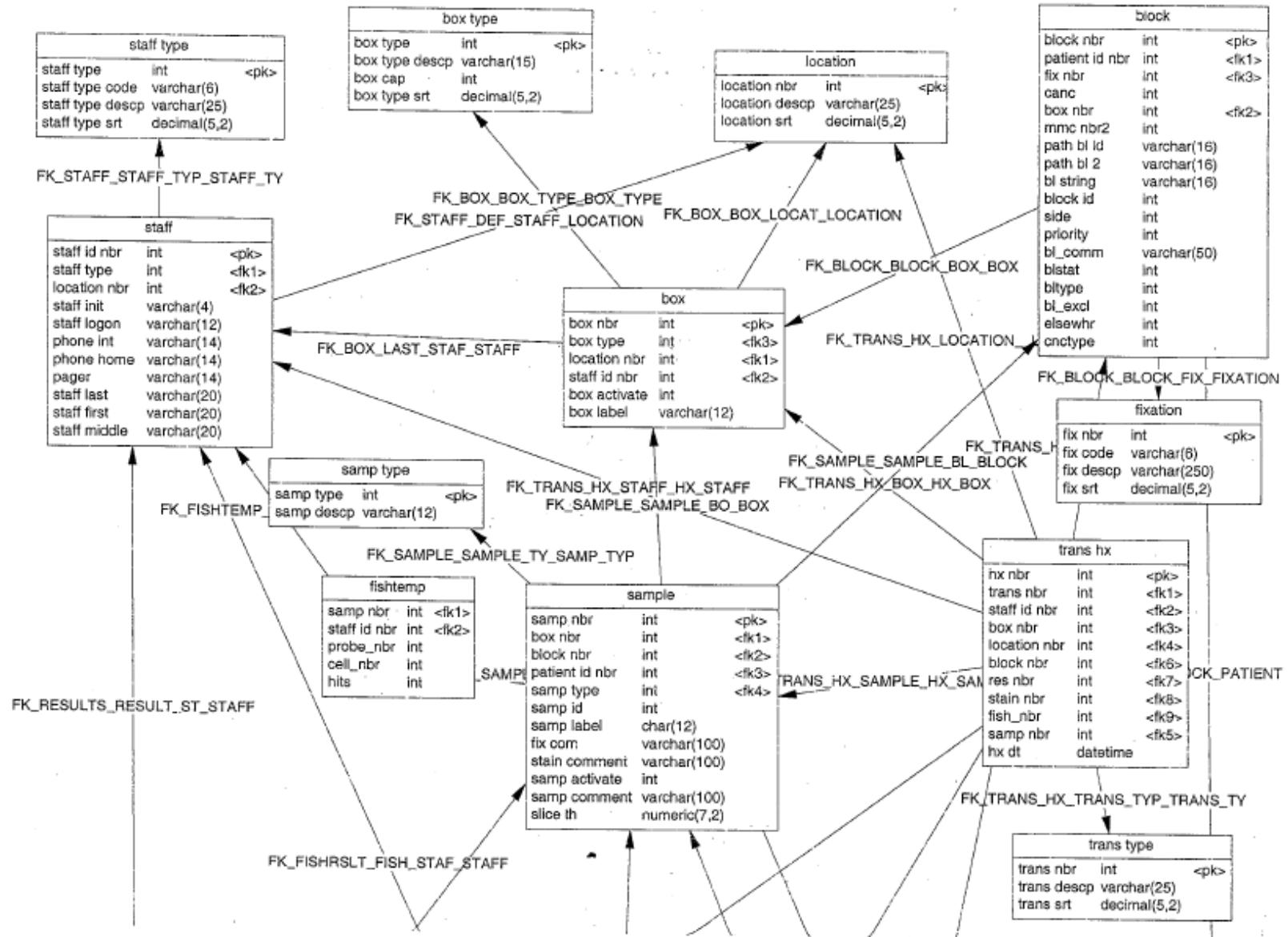
are done to all samples in the boxes. Each activity is stored in the database along with the time of the activity and the identification number of the person who performed the activity.

At the time of sample analysis, the physician, researcher or laboratory technician scans the samples and uses the same web-based application to enter the results of the analysis. All results and transactional information are stored in the database and available for statistical analysis.

The database server software is the current Sybase relational database management system. The data model was created and managed with Sybase PowerDesigner. The users will access the database via the Mayo internal web using programs written in Cold Fusion. The data analysts will access the database using connections to the SAS statistics analysis system. ODBC connections are used to connect web forms and the SAS system to the database.

The components of the database are pictured in the figure below. The central table in the database is the "sample" table which contains links to most of the other tables such as the "patient" table (containing patient information), the "block" table (containing information about the paraffin block from which the sample was cut), the "box" table (showing the current location of the sample), the "stain" table (showing stains done to the sample), the "results" table (containing the results of many types of tests done to each sample) and the "fishrslt" table (a table for FISH results). Most of these secondary tables contain links to descriptive tables, such as the "location" table and the "test\_type" table, which manage the drop-down selection boxes in the web pages.

Figure 18: Relational database



## VII. Key research accomplishments

- We created a retrospective cohort of women with BBD that can support tissue-based risk prediction strategies. Tissue-based features that are strongly associated with risk of breast cancer may signal processes and/or mediators that are central to the process of breast carcinogenesis.
- We identified the degree of risk associated with the common benign epithelial entities and the extent to which age at biopsy and family history influence the risk of breast cancer in women with proliferative or atypical lesions. The highest risk was among women who had proliferative disease with atypia, especially those of younger age (Hartmann et al., NEJM, 2005).
- We identified a marked increased risk of breast cancer in women with three or more foci of atypia, especially for three or more foci with calcifications. Also, risk was higher in women diagnosed with atypical hyperplasia before age 45. Among women with atypia, risk was not affected by family history (Degnim et al, JCO, 2007).
- We identified that a single papilloma without atypia imparts an increased risk of developing a subsequent carcinoma similar to other forms of proliferative breast disease without atypia. Atypical papilloma, particularly in the setting of multiple papillomas, imparts a breast cancer risk similar to or greater than conventional atypical ductal/lobular hyperplasias (Lewis et al, Am J Surg Pathol, 2006).
- We identified that the extent of lobular involution in breast tissue is an important risk indicator for the development of breast cancer. Increasing degrees of involution result in a significant reduction in breast cancer risk, even in women at “high risk” based on atypia or young age (Milanese et al., JNCI, 2006).
- We found that intense COX-2 expression is associated with a significantly greater likelihood of a subsequent breast cancer in women with atypia and represents one potential molecular target for chemoprevention strategies (Visscher et al., JNCI, 2008).
- We found no increased breast cancer risk for women with radial scars compared to the risk already present due to proliferative disease with or without atypia (Berg et al., Breast Cancer Res Treat, 2008).
- We identified that centrosome amplification is seen more frequently in higher risk benign lesions (e.g. atypia) and is infrequently seen in non-proliferative lesions and in proliferative lesions without atypia (Lingle et al., American Association for Cancer Research, 2005).
- We found the Gail model to predict no better than chance alone the breast cancer risk of women with atypia. The model significantly underestimated lifetime risk of our cohort of women with atypia (Pankratz et al., JCO, 2008).

- We found Ki67, a proliferation marker, to be predictive of risk after a benign breast biopsy. Those women who had higher proliferation rates were more likely than those with low proliferation to develop breast cancer within the first 10 years after benign biopsy. Conversely, if there is low proliferation, breast cancers tend to occur later, after 10 years of follow-up (Manuscript under review).
- We have helped to develop a retrospective cohort of African-American women with BBD at Wayne State University. Our findings thus far are that African-American women are younger at diagnosis and have more nonproliferative and mass forming lesions than Caucasian-American women. Additionally, lobular involution appears to occur at a slower rate in African-American women than Caucasian-American women (United States and Canadian Academy of Pathology, 2009).
- We found that measurements of lobular involution are highly consistent across multiple areas of a woman's breast (Vierkant et al., *Breast Cancer Res Treat*, 2008).
- We developed more quantitative measurements of lobular involution status to provide more objective assessment methods and to more precisely identify the extent of involution (McKian et al., *JCO*, 2009).
- To continue our work, we received an R01 fall 2008: *Risk prediction for breast cancer: a tissue based strategy* which builds on the Center of Excellence work. The R01 funding is for 5 years.

### **VIII. Reportable outcomes**

#### **A. Presentations**

##### **Poster presentation at the annual meeting of the American Association for Cancer Research, April 2003**

- Hartmann LC, Visscher D, Reynolds C, Frost MH, Melton LJ, Vachon C, Tlsty T, Hillman D, Johnson JL, Lingle WL, Suman V, Sellers TA. Benign breast disease and breast cancer risk.

##### **Poster presentation at the annual meeting of the American Association for Cancer Research, Orlando, FL, March 2004**

- Hartmann LC, Visscher D, Frost MH, Melton LJ, Vachon C, Couch F, Shridhar V, Ghosh K, Degnim A, Hillman D, Suman V, Vierkant RA, Maloney SD, Pankratz VS, Tlsty T, Sellers TA, Lingle WL. Benign breast disease and breast cancer risk.

##### **Podium presentation at annual meeting of the United States and Canadian Academy of Pathology. February 29, 2005 in San Antonio, Texas**

- Lewis, JT, Vierkant RA, Maloney SD, Hartmann LC, Visscher DW. Analysis of cancer risk among patients with papillary lesions of the breast

**Podium presentation at Society of Surgical Oncology Annual Cancer Symposium, March 3-6, 2005 in Atlanta, Georgia**

- Degnim, AC, Visscher D, Frost MH, Melton LJ, Vierkant RA, Maloney SD, Pankratz VS, Sellers TA, Lingle WL, Hartmann LC. Multifocal atypia confers increased risk of breast cancer

**Poster presentation at annual meeting of American Association for Cancer Research, April 16-20, 2005 in Anaheim, California**

- Hartmann LC, Sellers TA, Frost MH, Lingle WL, Degnim AC, Ghosh K, Vierkant RA, Maloney SD, Pankratz VS, Hillman DW, Suman VJ, Johnson J, Blake C, Tlsty T, Vachon CM, Melton LJ, Visscher DW. Benign breast disease and breast cancer risk in the Mayo Cohort Study

**Symposium and poster presentations at the Department of Defense Era of Hope June 10, 2005 in Philadelphia, Pennsylvania**

- Degnim AC, Visscher D, Frost MH, Melton LJ, Vierkant RA, Maloney SC, Pankratz VS, Sellres TA, Lingle WL, Tlsty T, Berman H, Hartmann LC. Multifocal atypia confers increased risk of breast cancer
- Ghosh K, Hartmann LC, Sellers TA, Degnim AC, Pankratz VS, Blake C, Tlsty T, Melton LJ, Visscher DW. Temporal changes in benign breast disease 1967 to 1991
- Hartmann LC, Frost MH, Ghosh K, Degnim A, Vierkant RA, Maloney SD, Pankratz VS, Tlsty T, Blake C, Sellers TA, Lingle WL, Melton J, Visscher DW. Benign breast disease and breast cancer risk
- Hartmann LC, Degnim A, Frost MH, Vierkant RA, Maloney SD, Sellers, TA, Pankratz VS, Tlsty T, Blake C. Lingle WL, Visscher DW. Benign breast disease: evidence for precursor lesions
- Pankratz VS, Vierkant RA, Maloney SD, Degnim AC, Hartmann LC. Statistical methods to assess the timing and side of breast cancer relative to benign breast biopsies: implications for potential precursor lesions

**Podium presentation at annual meeting of the United States and Canadian Academy of Pathology, Atlanta, GA, Feb. 11-17, 2006**

- Milanese TR, Hartman LC, Vierkant RA, Maloney SD, Frost MH, Pankratz VS, Visscher DW. The impact of lobular involution on breast cancer risk.

**Poster presentation at annual meeting of the United States and Canadian Academy of Pathology, Atlanta, GA, Feb. 11-17, 2006**

- Berg JC, Lewis JT, Maloney SD, Vierkant RA, Hartmann LC, Visscher DW. Analysis of cancer risk in women with radial scars of the breast.

**Podium presentation at annual meeting of American Association for Cancer Research, Washington, D.C., April 1-5, 2006**

- Hartmann LC, Lingle WL, Frost MH, Maloney SD, Vierkant RA, Pankratz VS, Tlsty T, Degnim AC, Visscher DW. COX-2 expression in atypia: Correlation with breast cancer risk.

**Poster presentation at American Association of Cancer Research, Washington DC, April 1-5, 2006.**

- Pankratz VS, Vierkant RA, Maloney SD, Frost MH, Visscher DW, Hartmann LC. Assessment of the Gail model in a cohort of women with atypical hyperplasia.

**Poster presentation at Joint Statistical Meetings, Minneapolis, MN, August 10, 2006.**

- Pankratz VS, Vierkant SD, Maloney SD, Hartmann LC. Epidemiologic comparisons of disease incidence among populations: The person-years approach.

**Poster presentation at 29<sup>th</sup> Annual San Antonio Breast Cancer Symposium, San Antonio, TX, December 16, 2006.**

- Ghosh K, Hartmann LC, Maloney D, Vierkant RA, Milanese TM, Visscher DW, Pankratz VS, Vachon CM. Mammographic breast density is inversely associated with age-related involution.
- Hartmann LC, Milanese TR, Sellers TA, Frost MH, Vierkant RA, Maloney SD, Pankratz VS, Degnim AC, Vachon CM, Reynolds CA, Thompson RA, Melton LJ, Goode EL, Visscher DW. Age-related lobular involution and risk of breast cancer.

**Poster presentation at the annual meeting of the American Association for Cancer Research, April 2005**

- Lingle W, Negron V, Bruzek A, Murphy L, Riehle D, Vierkant RA, Pankratz VS, Hartmann LC, Visscher DW. Centrosome amplification is greatest in benign breast lesions associated with an increase in risk of cancer.

**Poster presentation at American Society of Clinical Oncology's Breast Cancer Symposium, San Francisco, CA, September 2007.**

- Boughey JC, Hartmann LC, Degnim AC, Vierkant RA, Ghosh K, Vachon CM, Maloney SD, Reynolds C, Pankratz VS. Assessment of the accuracy of the Gail model in women with atypical hyperplasia.

**Poster presentation at Society of Clinical Oncologists Breast Cancer Symposium, Washington D.C., Spetember 6, 2008.**

- McKian KP, Reynolds CA, Vierkant RA, Anderson SS, Frost MH, Pankratz VS, Visscher DW, Nassar A, Hartmann LC. A novel, tissue-based feature strongly associated with breast cancer risk.

**Podium presentation at San Antonio Breast Cancer Symposium, San Antonio, TX, December 10-14, 2008.**

- Ghosh K, Pankratz VS, Reynolds CA, Vierkant RA, Anderson SS, Degnim AC, Visscher DW, Frost MH, Vachon CM, Hartmann LC. Benign breast disease and breast cancer risk in young women.

**Poster presentation at United States and Canadian Academy of Pathology Annual Meeting, Boston, MA, March 7-13, 2009.**

- Barr-Fritchler EG, Hartmann LC, Degnim AC, Anderson SS, Vierkant RA, Frost M, Visscher DW, Reynolds C. Estrogen receptor expression in atypical hyperplasia and its association with type of atypia and age.

**Podium presentation at United States and Canadian Academy of Pathology Annual Meeting, Boston, MA, March 2009.**

- Sharafeldeen B, Hayek K, Frost M, Hartmann L, Visscher D, Nassar. Benign breast disease in African-American women
- Visscher DW, Vierkant R, Reynolds C, Anderson S, Hartmann L. Clinical analysis of mucocele-like tumors of the breast: analysis of a large benign breast disease cohort.

***B. Manuscripts***

- Hartmann LC, Sellers TA, Frost MH, Lingle WL, Degnim AC, Ghosh K, Vierkant RA, Maloney SD, Pankratz VS, Hillman DW, Suman VJ, Johnson J, Blake C, Tlsty T, Vachon CM, Melton LJ, Visscher DW. Benign breast disease and the risk of breast cancer, *New England Journal of Medicine* 2005;353:229-37.
- Lewis JT, Hartmann LC, Vierkant RA, Maloney SD, Pandratz S, Allers TM, Frost MH, Visscher DW. An analysis of breast cancer risk in women with single, multiple, and atypical papilloma, *American Journal of Surgical Pathology* 2006;30:665-672.
- Milanese TR, Hartmann LC, Sellers TA, Frost MH, Vierkant RA, Maloney SD, Pankratz VS, Degnim AC, Vachon CM, Reynolds CA, Thompson RA, Melton LJ, Good EL, Visscher DW. Age-related lobular involution and risk of breast cancer, *Journal of the National Cancer Institute* 2006;98(22):1600-1607.
- Degnim AC, Visscher DW, Berman HK, Frost MH, Sellers TA, Vierkant RA, Maloney SD, Pankratz VS, deGroen PC, Lingle WL, Ghosh K, Penheiter L, Tlsty T, Melton LJ, Reynolds CA, Hartmann LC. Stratification of breast cancer risk in women with atypia: A Mayo cohort study, *Journal of Clinical Oncology* 2007;25(19):2671-7.
- Berg JC, Visscher DW, Vierkant RA, Pankratz VS, Maloney SD, Lewis JT, Frost MH, Ghosh K, Degnim AC, Brandt KR, Vachon CM, Reynolds CR, Hartmann LC. Breast cancer risk in women with radial scars in benign breast biopsies. *Breast Cancer Research and Treatment* 2008;108:167-174.
- Visscher DW, Pankratz VS, Santisteban M, Reynolds C, Ristimaki A, Vierkant RA, Lingle WL, Frost MH, Hartmann LC. Association between cyclooxygenase-2 expression in atypical hyperplasia and risk of breast cancer. *Journal of the National Cancer Institute* 2008;100(6):421-7.
- Pankratz VS, Hartmann LC, Degnim AC, Vierkant RA, Ghosh K, Vachon CM, Frost MH, Maloney SD, Reynolds C, Boughey JC. Assessment of the accuracy of the

Gail Model in women with atypical hyperplasia. *Journal of Clinical Oncology* 2008;26(33):5374-9.

- Vierkant RA, Hartmann LC, Pankratz VS, Anderson SS, Radisky D, Frost MH, Vachon CM, Ghosh K, Distad TJ, Degnim AC, Reynolds CA. Lobular involution: localized phenomenon or field effect? *Breast Cancer Research and Treatment* 2008; e-pub ahead of print.
- McKian KP, Reynolds CA, Visscher DW, Nassar A, Radisky DC, Vierkant RA, Degnim AC, Boughey JC, Ghosh K, Anderson SS, Minot D, Caudill JL, Vachon CM, Frost MH, Pankratz VS, Hartmann LC. A Novel Breast Tissue Feature Strongly Associated with Risk of Breast Cancer 2009 Accepted for publication JCO

### Conclusions

The Center of Excellence grant provided us the opportunity to establish a large cohort of women with BBD and to establish a tissue and data bank that includes benign tissue, subsequent breast cancer tissue, and clinical-epidemiologic data. We worked with Dr. Nassar to establish a cohort of African-American women at Wayne State University and with Dr. Tlsty's team at UCSF to advance basic discoveries. We were able to make some substantial contributions to our understanding of features present in breast tissue that are associated with later breast cancer. The most notable of these contributions were the identification of relative risk related to non-proliferative disease, proliferative disease without atypia and proliferative disease with atypia published in the *NEJM* and the significance of involution in relation to breast cancer risk published in *JNCI*. Our data showed that women with atypia have a relative risk of 4.24 compared to age-matched women from the Iowa SEER database. Novel data about involution was particularly striking because of its strong relationship with subsequent breast cancer and the fact that it can be determined using a single slide of tissue. We identified several biomarkers of notable significance in predicting breast cancer risk, including COX-2 and Ki-67. We successfully grew fresh breast tissue and used that tissue to identify new, potential markers of interest. We also successfully profiled tissue that had been stored for up to 25 years in paraffin. We contrasted histology findings between African-American and Caucasian-American women, finding involution to occur at a slower rate in African-American women. We examined the usefulness of the Gail model for women with atypical hyperplasia. We found this model to underestimate the risk of breast cancer in women with atypia. On an individual basis, the Gail model performed no better than chance alone in women with atypia. We successfully obtained an R01 for further risk prediction work. The combination of findings obtained through the Center of Excellence grant provides rich data for future research.

## Appendices

**Appendix A: Persons receiving pay from the research effort**

**Collaborators**

Lynn C. Hartmann, M.D., P.I.  
Thomas Sellers, Ph.D.  
Piet De Groen, M.D.  
Robert Jenkins, M.D., Ph.D.  
Aziza Nassar, M.D.  
Carol Reynolds, M.D.  
Daniel Visscher, M.D.  
Fergus Couch, Ph.D.  
Wilma Lingle, Ph.D.  
Viji Shridhar, Ph.D.  
Thea Tlsty, Ph.D., University of California, San Francisco  
Hind Nassar, M.D, Wayne State University, Johns Hopkins  
Rouba Ali-Fehmi, Wayne State University

**Statistical support**

Vera Suman, Ph.D.  
Vernon Pankratz, Ph.D.  
Rob Vierkant  
David Hillman  
Shaun Maloney  
Stephanie Anderson

**Data abstractors, study coordinators, data managers**

Marlene Frost, Ph.D.,  
Teresa Allers  
Betty Anderson, R.N.  
Mary Campion, R.N.  
Joanne Johnson, R.N.  
Melanie Kasner  
Margie Loprinzi, R.N.  
Lois Penheiter, R.N.  
Romayne Thompson  
Joel Worra

## **Appendix B: Abstracts of presentations**

Poster Presentation: American Association for Cancer Research, Annual Meeting, 2003.

**Benign Breast Disease and Breast Cancer Risk.** LC Hartmann<sup>1</sup>, D Visscher<sup>1</sup>, C Reynolds<sup>1</sup>, MH Frost<sup>1</sup>, LJ Melton<sup>1</sup>, C Vachon<sup>1</sup>, T Tlsty<sup>2</sup>, D Hillman<sup>1</sup>, JL Johnson<sup>1</sup>, WL Lingle<sup>1</sup>, V Suman<sup>1</sup>, TA Sellers<sup>1</sup>.

<sup>1</sup> Mayo Clinic Cancer Center, Rochester, MN

<sup>2</sup> University of California, San Francisco, CA

**Introduction:** Benign breast disease (BBD) is an established risk factor for breast cancer (BC), but only a minority of women with BBD ultimately develop BC. The ability to identify the subset of women at greatest risk for breast cancer at the time of BBD diagnosis would permit more aggressive clinical intervention, including closer surveillance and prevention opportunities. To facilitate this discovery process, we have established a large historical cohort of women with BBD in which we can test more specific means of risk prediction, using clinical, histopathologic and molecular tools.

**Methods:** The Mayo Clinic Surgical Index was used to identify all women who had an open breast biopsy with benign findings at the Mayo Clinic between 1/1/82 and 12/31/91 (n = 5153). The availability of tissue slides and blocks on these patients was verified through linkage to the Pathology Index. Medical record review was performed to verify eligibility and to identify subsequent occurrences of breast cancer diagnosed or treated at Mayo. A study-specific questionnaire was mailed to collect risk factor data on the cohort and to identify breast cancers diagnosed outside of Mayo.

**Results:** This 10-year cohort includes 5153 women with 66,290 person years of follow-up (through 2/02). The median age at BBD diagnosis was 54 years (13-94), and 41% were age 50 or less. Some family history of breast cancer was present in 32%, while 17% had an affected first-degree relative. Thus far, 255 women are known to have developed BC. The interval from BBD to BC is: ≤ 5 yrs, 33.7%; 5.1-10 yrs, 34.5 %; 10.1-15 yrs, 27.5%; > 15 yrs, 4.3%. The cancer occurred in the same breast as the BBD in 125 women (49%), the opposite breast in 84 (32.9%), and both breasts in 10 (3.9%). Side of BC is pending for 36 (14%) women. The estimated 5-year, 10-year and 15-year breast cancer incidence rates are 1.8% (95%CI: 1.4-2.1%), 3.6% (95%CI: 3.1-4.2%), and 5.8% (95%CI: 5.1-6.5%), respectively. Incorporating time from BBD to cancer and the side of BBD vs BC, we are exploring a panel of biomarkers as indicators of possible BC precursors or a background field change.

**Conclusions:** We have assembled a large cohort of patients with BBD with extensive follow-up for breast cancer, excellent participation on a risk factor survey, and sufficient quantities of well-characterized tissues to permit independent evaluation of established and novel molecular markers.

Supported by grants from the national Komen Foundation, the Breast Cancer Research Foundation, and DOD Breast Cancer Center of Excellence award DAMD 17-02-1-0473.

Poster Presentation: American Association for Cancer Research, Annual Meeting, March 27-31, 2004, Orlando, FL.

**Benign Breast Disease and Breast Cancer Risk.** LC Hartmann<sup>1</sup>, D Visscher<sup>1</sup>, MH Frost<sup>1</sup>, LJ Melton<sup>1</sup>, C Vachon<sup>1</sup>, F Couch<sup>1</sup>, V Shridhar<sup>1</sup>, K Ghosh<sup>1</sup>, A Degnim<sup>1</sup>, D Hillman<sup>1</sup>, V Suman<sup>1</sup>, RA Vierkant<sup>1</sup>, SD Maloney<sup>1</sup>, VS Pankratz<sup>1</sup>, T Tlsty<sup>2</sup>, TA Sellers<sup>3</sup>, WL Lingle<sup>1</sup>.

<sup>1</sup>Mayo Clinic Cancer Center, Rochester, MN

<sup>2</sup>University of California, San Francisco, CA

<sup>3</sup>Moffitt Cancer Center, Tampa, FL

**Introduction:** Benign breast disease (BBD) is an established risk factor for breast cancer (BC), but only a minority of women with BBD ultimately develop BC. To identify the subset of women at greatest risk for breast cancer at the time of BBD diagnosis, we have established a large historical cohort of women with BBD in which we can test more specific means of risk prediction, using clinical, histopathologic and molecular tools.

**Methods:** The Mayo Clinic Surgical Index was used to identify all women ages 18-85 who had an open breast biopsy with benign findings at the Mayo Clinic between 1/1/67 and 12/31/91. The availability of tissue slides and blocks on these patients was verified through linkage to the Pathology Index. Medical record review was performed to verify eligibility and to identify subsequent occurrences of breast cancer diagnosed or treated at Mayo. A study-specific questionnaire is being used to collect risk factor data on the cohort and to identify breast cancers diagnosed outside of Mayo.

**Results:** This 25-year cohort includes 11,782 women with 181,284 person years of follow-up. The median age at BBD diagnosis was 50.0 years. Some family history of BC was present in 40% of those surveyed; 21% had an affected first-degree relative. Thus far, 705 women are known to have developed BC, at a median of 9.2 years after their BBD. The interval from BBD to BC is <= 5 years, 27%; 5.1 - 10 years, 26%; 10.1-15 years, 24%; >15 years, 23%. The cancer occurred in the same breast as the BBD in 279 women (40%), the opposite breast in 189 (27%) and both breasts in 18 (3%). Side is pending for 219 (31%). The estimated 5-yr, 10-yr and 15-yr BC incidence rates are 2.0%, 4.1%, and 6.4%, respectively for women with BBD from 1982-1991 (follow-up ongoing for 1967-81 group). The histopathologic review has been completed for 3,004 of the BBD specimens. Non-proliferative disease was found in 65.8%, proliferative disease without atypia in 28.6% and atypia (atypical ductal hyperplasia or atypical lobular hyperplasia) in 3%. Incorporating time from BBD to BC, histology, and side of BBD vs BC, we are exploring a panel of biomarkers as indicators of possible BC precursors or a background field change.

**Conclusions:** We have assembled a large cohort of patients with BBD with extensive follow-up for breast cancer, excellent participation on a risk factor survey, and sufficient quantities of well-characterized tissues to permit independent evaluation of established and novel molecular markers.

Supported by grants from the national Komen Foundation, the Breast Cancer Research Foundation, and DOD Breast Cancer Center of Excellence award DAMD 17-02-1-0473.

**Analysis of Cancer Risk among Patients with Papillary Lesions of the Breast**  
*JT Lewis, RA Vierkant, SD Maloney, LC Hartmann, DW Visscher. Mayo Clinic, Rochester, MN*

**Background:** Papillomas are relatively common breast lesions. Although most are single and histologically bland, they may be multiple and demonstrate varying degrees of atypia. The risk of breast carcinoma development in patients with benign papillary breast lesions is incompletely defined.

**Design:** Papillary breast lesions were identified in a histopathologically-defined benign breast disease cohort of 8872 patients biopsied between 1967-1991. Cases were subclassified into four groups: single papilloma without atypia, single papilloma with atypia, multiple ( $>3$ ) papillomas without atypia, and multiple papillomas with atypia. Using Cox proportional hazards regression, the risk of cancer development among these groups was compared to patients with other forms of proliferative breast disease (with or without atypia) and patients with non-proliferative breast changes.

**Results:** Of the 368 patients diagnosed with a single papilloma without atypia, 35 (10%) developed carcinoma. Eleven (22%) of the 49 women with a single papilloma with atypia subsequently developed carcinoma. Forty-one patients were diagnosed with multiple papillomas without atypia, and six (15%) developed carcinoma. Twelve cases of multiple papillomas with atypia were identified, and 4 (33%) of these developed carcinoma. The relative risk of cancer development is presented in Table 1.

**Conclusions:** We conclude that the diagnosis of a single papilloma without atypia imparts an increased risk of developing a subsequent carcinoma similar to other non-atypical forms of proliferative breast disease. Atypical papilloma, particularly in the setting of multiple papillomas, imparts a breast cancer risk similar to or greater than conventional atypical ductal/lobular hyperplasias.

Incident Breast Cancer Relative Risk		
Diagnosis (N)	Person Years Follow-up	Relative Risk (95% CI)
Non-Proliferative (5934)	91129	1.00
Proliferative without Atypia (2211)	32895	1.60 (1.35, 1.90)
Proliferative with Atypia (257)	3127	3.59 (2.63, 4.92)
Single Papilloma without Atypia (368)	4979	1.82 (1.28, 2.58)
Single Papilloma with Atypia (49)	577	4.88 (2.67, 8.92)
Multiple Papillomas without Atypia (41)	592	2.81 (1.25, 6.31)
Multiple Papillomas with Atypia (12)	115	8.66 (3.22, 23.31)

Relative risks were calculated using a Cox proportional hazards regression analysis. Results are adjusted for age.

**Multifocal atypia confers increased risk of breast cancer.**

Degnim AC, Visscher D, Frost M, Melton L, Vierkant R, Maloney S, Pankratz V, Seller T, Lingle W, Hartmann L.

**Introduction:** We evaluated breast cancer risk in relation to histologic features of atypia in a large retrospective cohort of women with benign breast disease.

**Methods:** Through surgical and pathology indexes, women were identified who had an open breast biopsy with benign findings at our institution between 1/1/67 and 12/31/91. Histologic review of original biopsy slides or tissue blocks was performed by a single pathologist who was blinded to clinical outcome. Histopathologic information was collected, including type of hyperplasia and number of atypical foci. Subsequent breast cancers were identified through a follow-up survey and our Tumor Registry.

**Results:** Of 11,109 eligible women with benign breast biopsies, histologic review and follow-up are completed on 9,874 to date. Of 311 biopsies demonstrating atypical hyperplasia, 43% had only atypical ductal hyperplasia (ADH), 52% had only atypical lobular hyperplasia (ALH) and 4% had both ADH and ALH. Data on number of atypical foci are complete for 300 patients; of these, 58% had one focus of atypia, 26% had two foci, and 16% had 3 or more foci. With 3702 person-years of follow-up in these 311 women, we observed 60 cases of incident breast cancer. Age-adjusted Cox proportional hazard regression analysis showed a significantly increased relative risk (RR) of breast cancer with any atypia compared to 5950 women with non-proliferative disease (NPD) without atypia (see Table), and a trend toward higher risk with ADH versus ALH.

Similar analysis showed a significantly increased risk of breast cancer with increasing number of atypical foci (see Table). Women with 3 foci of atypia had an 8-fold greater risk of breast cancer compared to those with NPD ( $p<0.01$ ) and a three-fold greater risk compared to those with one focus of atypia ( $p<0.01$ ).

**Conclusions:** In women with atypia on benign breast biopsy, multiple foci of atypia indicate a significantly higher risk of subsequent breast cancer. Ductal atypia may convey a somewhat greater risk of subsequent breast cancer compared to ALH.

Variable	Description	Events	RR (95% CI)	p-value
Histologic Type	Non-Prol Dis W/O Atypia	344	1.0 (Reference)	<0.01
	ALH	30	3.6 (2.5, 5.3)	
	ADH	26	4.5 (3.0, 6.7)	
	ALH and ADH	4	5.8 (2.2, 15.6)	
# of Foci of Atypia	Non-Prol Dis W/O Atypia	344	1.0 (Reference)	p<0.01
	1 focus of atypia	21	2.5 (1.6, 4.0)	
	2 foci of atypia	18	4.9 (3.0, 7.9)	
	3 or more foci of atypia	19	8.2 (5.1, 13.0)	

### Benign Breast Disease and Breast Cancer Risk in the Mayo Cohort Study

Lynn C. Hartmann<sup>1\*</sup>, Thomas A. Sellers<sup>2</sup>, Marlene H. Frost<sup>1\*</sup>, Wilma L. Lingle<sup>1<sup>a</sup></sup>, Amy C. Degnim<sup>1<sup>b</sup></sup>, Karthik Ghosh<sup>1<sup>c</sup></sup>, Robert A. Vierkant<sup>1<sup>d</sup></sup>, Shaun D. Maloney<sup>1<sup>d</sup></sup>, V. Shane Pankratz<sup>1<sup>d</sup></sup>, David W. Hillman<sup>1<sup>d</sup></sup>, Vera J. Suman<sup>1<sup>d</sup></sup>, Jo Johnson<sup>1\*</sup>, Cassann Blake<sup>1\*</sup>, Thea Tlsty<sup>4</sup>, Celine M. Vachon<sup>1<sup>e</sup></sup>, L. Joseph Melton III<sup>1<sup>e</sup></sup>, Daniel W. Visscher<sup>1<sup>e</sup></sup>. Mayo Clinic College of Medicine, 200 First Street SW, Rochester, MN 55905 (\*Division of Medical Oncology; <sup>a</sup>Division of Experimental Pathology; <sup>b</sup>Division of General Surgery; <sup>c</sup>Division of General Internal Medicine; <sup>d</sup>Division of Biostatistics; <sup>e</sup>Division of Epidemiology; <sup>f</sup>Division of Anatomic Pathology); <sup>2</sup>H. Lee Moffitt Cancer Center & Research Institute, 12902 Magnolia Drive, Tampa, FL 33620; <sup>3</sup>Wayne State University, Detroit MI 48202; <sup>4</sup>University of California, San Francisco, 513 Parnassus Avenue, San Francisco, CA. 94143.

**Background:** Benign breast disease is a significant risk factor for breast cancer. To optimize management of these women, we need precise risk estimates for them. Moreover, it remains unclear if these lesions represent precursors or generalized risk indicators.

**Methods:** We identified all women with benign breast disease at the Mayo Clinic between 1967 and 1991. Breast cancer events and risk factors were obtained from the medical record and questionnaires. All benign specimens were reviewed by our breast pathologist. To estimate relative risks, we compared observed breast cancers to those expected using Iowa SEER rates.

**Results:** This Mayo cohort includes 9,087 women followed for a median of 15 years. The histologies are: non-proliferative (66%); proliferative without atypia (30%) and atypical hyperplasia (4%). 707 breast cancers have developed to date. The relative risk for the cohort is 1.56 (95% CI 1.45-1.68). Increased risk persisted to at least 25 years after biopsy. Atypia conveyed a RR of 4.24 (95% CI 3.26-5.41), vs 1.88 for proliferative changes without atypia and 1.27 (95% CI 1.15-1.41) for non-proliferative lesions. Family history information was available for 4,808 women (53%) and conveyed risk independent of histology, with a RR of 1.93 for a strong family history (95% CI 1.58-2.32) vs. 1.18 (95% CI 1.01-1.37) for no family history. For women with negative family history and non-proliferative findings, we saw no increased risk. In the first 10 years after benign biopsy, an excess of cancers occurred in the same breast, especially in women with atypia, consistent with the presence of precursor lesions.

**Conclusions:** Proper categorization of women with benign breast disease can differentiate high risk subsets from those at no increased risk.

Symposium and Poster Presentations: Department of Defense Era of Hope, June 9 -10, 2005, Philadelphia, PA

## MULTIFOCAL ATYPIA CONFERS INCREASED RISK OF BREAST CANCER

AC Degnim<sup>1</sup>, D Visscher<sup>1</sup>, MH Frost<sup>1</sup>, LJ Melton<sup>1</sup>, RA Vierkant<sup>1</sup>, SD Maloney<sup>1</sup>, VS

Pankratz<sup>1</sup>, TA Sellers<sup>2</sup>, WL Lingle<sup>1</sup>, T Tlsty<sup>3</sup>, H Berman<sup>3</sup>, LC Hartmann<sup>1</sup>

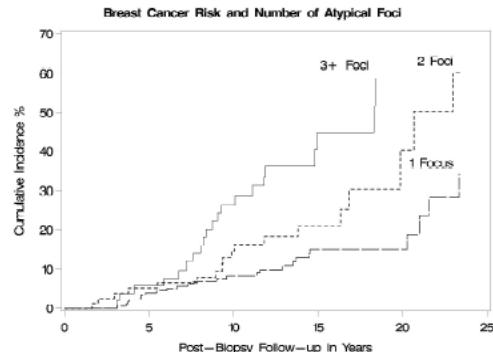
<sup>1</sup>Mayo Clinic Cancer Center, <sup>2</sup>Moffitt Cancer Center, <sup>3</sup>University of California San Francisco  
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We evaluated breast cancer (BC) risk in relation to histologic features of atypia in a large retrospective cohort of women with benign breast disease.

Through surgical and pathology indexes, women were identified who had a surgical breast biopsy with benign findings at our institution between 1/1/67 and 12/31/91. Histologic review of original biopsy slides or tissue blocks was performed by a single pathologist (DV) who was blinded to clinical outcome. Histopathologic information was collected, including type of hyperplasia and number of atypical foci. Subsequent BCs were identified through a follow-up survey and our Tumor Registry. We compared the observed number of incident BCs among women in our cohort with atypical hyperplasia (AH) to that expected using incidence rates from the Iowa SEER data. Internal comparisons of BC and number of atypical foci were made using Cox proportional hazards regression.

Of 10,032 eligible women with benign breast biopsies, histologic review and follow-up are completed on 9,087. Of 336 biopsies demonstrating AH, 62% had one focus of atypia, 24% had two foci, and 14% had 3 or more foci. We observed 4161 person-years of follow-up and 64 cases of incident BC in these 336 women. Those with AH had a 4.2-fold increased risk of BC compared to Iowa SEER (95% CI 3.3-5.4). Kaplan-Meier estimates show increasing risk of BC with increasing number of atypical foci (Figure 1). Women with 3 foci of atypia had a 9.4-fold increased risk of BC vs. Iowa SEER and a 4.1-fold greater risk vs. those in the cohort with one focus of atypia ( $p<0.01$  for each).

In women with AH on benign breast biopsy, multiple foci of atypia indicate a significantly higher risk of subsequent BC. These results may impact decision-making in women with AH who are at increased risk for BC.



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**TEMPORAL CHANGES IN BENIGN BREAST DISEASE 1967 TO 1991**

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**Background:** Women with benign breast disease (BBD) are at increased risk of breast cancer (BC). The classic study of BBD by Dupont and Page enrolled women with biopsies in the 1950s-1960s. We sought to assess changes in the nature of BBD over time, utilizing a 25-year cohort of BBD from the late 1960s to the early 1990s.

**Methods:** Utilizing the Mayo Clinic Surgical and Pathology Indices, women ages 18 to 85 who had benign excisional breast biopsy between January 1, 1967 and December 31, 1991 were identified. The clinical outcome of BC was the end-point for follow-up for the 'cases' and was determined using the Mayo medical record and questionnaire information sent to study participants. Our breast pathologist (DV), blinded to both the initial diagnosis and clinical outcome, performed pathology review.

**Results:**

Characteristic	All Subjects	Year of Biopsy				
		1967-71	1972-76	1977-81	1982-86	1987-91
<b>Overall</b>	9087 (100%)	971 (10.7%)	1808 (19.9%)	1509 (16.6%)	2295 (25.3%)	2504 (27.6%)
<b>Breast Cancer Status</b>						
Case	707 (7.8%)	122 (12.6%)	190 (10.5%)	112 (7.4%)	138 (6%)	145 (5.8%)
<b>Mean Age at Biopsy</b> (standard deviation)	51.4 (14.3)	47.5 (13.2)	49.3 (13.4)	48.8 (14.3)	53.5 (14.3)	54.1 (14.4)
<b>Histology</b>						
Non-Prolif. Dis	6061 (66.7%)	713 (73.4%)	1339 (74.1%)	1057 (70%)	1469 (64%)	1483 (59.2%)
Prolif. Dis. W/O Atypia	2690 (29.6%)	245 (25.2%)	435 (24.1%)	411 (27.2%)	728 (31.7%)	871 (34.8%)
Prolif. Dis. W/Atypia	336 (3.7%)	13 (1.3%)	34 (1.9%)	41 (2.7%)	98 (4.3%)	150 (6%)
<b>Family History</b>						
Known	4808	258	601	530	1572	1847
Negative	2668 (55.5%)	126 (48.8%)	270 (44.9%)	282 (53.2%)	899 (57.2%)	1091 (59.1%)
Weak	1174 (24.4%)	70 (27.1%)	172 (28.6%)	114 (21.5%)	384 (24.4%)	434 (23.5%)
Strong	966 (20.1%)	62 (24%)	159 (26.5%)	134 (25.3%)	289 (18.4%)	322 (17.4%)

**Benign Breast Disease by 5-year Interval**

**Conclusions:** This study provides data regarding the changing nature of BBD. The number of women in each 5-year period increased, likely due to growth of clinical practice at Mayo Clinic but may also reflect increased adoption of screening mammography. Within each time-frame, there were over 100 cases of BC, but the proportion of 'cases' to 'non-cases' decreased with decreasing 'years of risk' for women in the latter part of the study. Mean age at biopsy increased from 47.5 to 54.1, and BBD samples from the latter years of the study were more likely to show proliferative change with or without atypia, again likely due to increased use of screening mammography and detection of abnormal calcifications. The stable proportion of women with positive family history (about 20% of whom had a strong family history) is consistent with general breast cancer awareness and screening practices in this population.

## BENIGN BREAST DISEASE: EVIDENCE FOR PRECURSOR LESIONS

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**Background:** Benign breast disease (BBD) represents a significant risk factor for a later breast cancer (BC) that can occur in either breast. Besides aiding in risk prediction, BBD provides a possible window into a continuum of alterations culminating in BC. Information about time to and side of BC after BBD has not been available for most prior studies of BBD. Such information can help distinguish possible precursor lesions from markers of increased risk.

**Methods:** We used the Mayo Clinic Surgical Index to identify women ages 18-85 who had BBD between 1-1-67 and 12-31-91. The benign H&E-stained sections were evaluated by our study pathologist (DV). Biopsies were classified into: 1) non-proliferative changes, 2) proliferative changes without atypia (PDWA), and 3) atypical hyperplasia (AH). To estimate relative risks, we compared the observed number of incident BCs in our cohort to that expected, using age- and calendar period-matched incidence rates from the Iowa SEER data as the reference.

**Results:** This cohort consists of 9087 women who have been followed for a median of 15 years (person years 144, 881). The benign histologies include: non-proliferative [n=6061 (66%)], PDWA [n=2690 (30%)] and AH [n=336 (4%)]. 707 breast cancers have occurred to date. The overall relative risk for breast cancer for the entire cohort is 1.56 (95% CI 1.45 – 1.68). Benign histology was a major predictor of risk. AH conveyed a relative risk of 4.24 (95% CI 3.26 – 5.41) vs 1.88 (1.66 – 2.12) for women with PDWA and 1.27 (1.15 – 1.41) for non-proliferative lesions. The table shows median years to BC and side of BC by histologic category for those women who developed BC. There is a greater tendency for BC to develop sooner (p=0.03) and in the ipsilateral breast in women whose BBD contained increasing degrees of proliferation and atypia—consistent with the presence of precursors in these higher risk entities.

**Conclusion:** Information about side of BC and time to BC in studies of BBD can help to identify probable precursor lesions. Studies based in these lesions can guide our understanding of molecular risk and molecular carcinogenesis.

### Sidedness and Timing of Breast Cancers after BBD

Benign Histology	# of BCs*	Median Yrs to BC (1st -3rd quartile)	Side of BC*	
			Same (n,%)	Opposite (n,%)
Non-proliferative	379	10.7 (5.4-16.4)	185 (54)	156 (46)
PDWA	264	11.0 (5.8-16.0)	123 (56)	96 (44)
AH	64	9.3 (5.7-14.5)	34 (61)	22 (39)

\* cancers where both BBD and BC were unilateral events and side for both was known

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**STATISTICAL METHODS TO ASSESS THE TIMING AND SIDE OF BREAST CANCER RELATIVE TO BENIGN BREAST BIOPSIES: IMPLICATIONS FOR POTENTIAL PRECURSOR LESIONS**

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**Introduction:** Benign breast disease is an important predictor of risk for breast cancer. It may also provide information about a continuum of benign breast alterations culminating in breast cancer. The agreement between side of the benign lesion and subsequent breast cancer provides one means of obtaining evidence for the presence of precursors. However, little data have been reported describing the concordance between side of the benign lesion and the cancer. Also, methods to assess the evidence of this concordance, particularly with regard to the time interval between benign lesion and breast cancer are lacking.

**Methods:** Extensive follow-up data were obtained from a consecutive series of women undergoing an open breast biopsy with benign findings from 1967 through 1991, including the timing of subsequent breast cancers and the side(s) of benign biopsy and cancer development. A variety of methods to assess concordance between benign lesions and breast cancers were explored. These ranged from the simple (e.g. chi-square tests) to the complex (e.g. survival models). Ultimately, we estimated the relative risk of cancer in the same vs. the opposite breast for five-year time intervals using a survival analysis approach by computing the relative incidence of ipsilateral and contralateral cancers. We calculated the incidence for each of these categories using two observations per person and censoring for the type of cancer that did not occur. Using this method, the relative risks are equivalent to ratios of observed events, as the approach yields identical person years for each event type. We capitalized on this and used properties of the binomial distribution to obtain exact p-values and 95% confidence intervals for these relative risks.

**Results:** The study has so far followed 9087 eligible women for 144,881 person-years (median 15 years), and 707 breast cancers have been observed to date. 91 of these cases were either missing side information, or had bilateral biopsies or cancers. Most of the unilateral events, 342 of 616 (56%), developed in the same breast as the benign biopsy. During the first ten years, there was an excess of ipsilateral cancers, with relative risks for ipsilateral vs. contralateral of 1.88 and 1.34 for years 0-5 and 6-10, respectively. Additionally, the 35 women with atypia who developed breast cancer within 10 years of their benign biopsy were 2.5 times more likely ( $p=0.02$ ) to develop cancer in the same breast vs. the opposite breast.

**Conclusions:** We have examined and used a range of statistical methods to evaluate side-specific breast cancer risk. An excess of breast cancers occurred in the same breast within the first years of follow-up, especially in women with atypia. This suggests that precursors may exist within the spectrum of benign breast disease that can be identified with molecular techniques and targeted with tailored interventions.

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## BENIGN BREAST DISEASE AND BREAST CANCER RISK

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**Background:** Benign breast disease (BBD) represents a significant risk factor for a later breast cancer that can develop in either breast. Questions remain about the degree of risk associated with non-proliferative findings and the degree of interaction between atypia and family history. Having accurate risk estimates is essential to counsel women properly regarding surveillance and risk reduction strategies.

**Methods:** The Mayo Clinic Surgical Index was used to identify all women ages 18-85 who had an open breast biopsy with benign findings at the Mayo Clinic between 1/1/67 and 12/31/91. Our study pathologist (DV) reviewed and classified all benign lesions. Medical records and a study-specific questionnaire were used to collect risk factor data and to identify subsequent breast cancers (BC). To estimate relative risks, we compared the observed number of incident BCs in our cohort to that expected, using age- and calendar period-matched incidence rates from the Iowa SEER data as the reference.

**Results:** This 25-year cohort includes 9,087 women with 144,881 person years of follow-up (median 15 yrs). The mean age at BBD was 51.4 years. Non-proliferative disease was found in 66%, proliferative disease without atypia in 30% and atypia (atypical ductal hyperplasia or atypical lobular hyperplasia) in 4%. Thus far, 707 women are known to have developed BC, at a median of 10.7 years after their BBD.

The overall relative risk for breast cancer in our cohort is 1.56 (95% CI 1.45 -1.68). Benign histology was a major predictor of risk. Atypical hyperplasia conveyed a relative risk of 4.24 (3.26 – 5.41) vs 1.88 (1.66 – 2.12) for women with proliferative disease without atypia and 1.27 (1.15 – 1.41) for non-proliferative lesions. Knowledge of family history allowed further refinement of risk estimates. For women with no family history, the relative risk was 1.18 (1.01 – 1.37) compared to 1.43 (1.15 – 1.75) for women with a weak family history, and 1.93 (1.58 – 2.32) for those with a strong family history. For women with non-proliferative findings and no or weak family history, there was no increased risk. We did not see an interaction between atypia and family history. Women with atypia and no family history had a RR of 2.95 (1.65 – 4.87) vs 4.18 (1.80 – 8.23) for those with a weak family history and 4.0 (2.07 – 6.99) for those with a strong family history. Risk of BC was inversely associated with age at benign biopsy, with younger women demonstrating greater risk than older women (RR for age < 30 = 1.83 vs RR 1.40 for age  $\geq$  70).

**Conclusions:** Benign breast disease is a major risk factor for a later breast cancer. Within BBD, age at BBD, family history and histology are major predictors of subsequent risk.

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Podium Presentation: United States and Canadian Academy of Pathology, Annual Meeting, February 11-17, 2006, Atlanta, GA.

**The Impact of Lobular Involution on Breast Cancer Risk.**

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**Background:** Lobular involution is a histologic finding that reflects atrophy associated with physiologic aging in the human breast. Based on epidemiologic associations, involution has been hypothesized to have relevance in breast tumorigenesis.

**Methods:** A breast pathologist examined benign breast biopsies of 8,743 women in the Mayo Benign Breast Disease cohort and classified them according to the degree of lobular involution as follows: none (0%), partial (1-74%), or complete (>75%). Each benign biopsy was also evaluated per standard criteria as nonproliferative (NP), proliferative disease without atypia (PDWA), and atypical hyperplasia (AH). Age at biopsy, family history of breast cancer, and development of breast cancer were obtained from medical records or questionnaires (17-year mean follow-up). Associations of involution with other breast cancer risk factors were carried out using chi-square tests and logistic regression analyses. Relative risks of breast cancer were estimated by comparing the number of observed events with the number expected based on rates from the Iowa SEER registry.

**Results:** Distribution of the patients by the three levels of involution was as follows: none-1,628 (18.6%); partial-5,202 (59.5%); and complete-1,913 (21.9%). Increased involution was found to correlate with increased age and decreased family history of breast cancer. The relative risk of breast cancer was significantly lower in patients who had complete (0.91, 95% CI 0.74-1.10) compared to those with partial (1.45, 95% CI 1.32-1.59) or no involution (1.88, 95% CI 1.59-2.21) ( $P<0.001$ ). Age and family history modified breast cancer risk. In patients with PDWA, the relative risk for women with no involution was (2.94, 95% CI 2.26-3.75), while that for women with complete involution was only (1.11, 95% CI 0.68-1.72) ( $P<0.001$ ). The relative risks in patients with NP and AH displayed similar associations.

**Conclusions:** The degree of lobular involution correlates inversely with breast cancer risk. It modifies breast cancer risk in patients stratified by age, family history, and type of histology. These data indicate that aberrant or delayed involution is a biologically important constitutional variable in breast cancer biology.

Poster Presentation: United States and Canadian Academy of Pathology, Annual Meeting, February 11-17, 2006, Atlanta, GA.

**Analysis Of Cancer Risk In Women With Radial Scars Of The Breast**

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**Background:** Radial scars (RS) are characterized by an elastotic central core containing entrapped tubules that radiate outward in a stellate manner. The epithelial component may show varying degrees of proliferation. Previous studies have shown that RS impart an increased risk of breast cancer development.

**Design:** RS were systematically identified in a histopathologically defined benign breast disease (BBD) cohort of 9073 patients biopsied between 1967 and 1991. Overall histology was classified as nonproliferative (NP), proliferative disease without atypia (PDWA), or atypical hyperplasia (AH) per standard criteria. The presence, number and size of RS were recorded for each case. The relative risk of cancer development within the BBD cohort was compared to that expected in the general population using standardized incidence ratios (SIRs).

**Result:** RS were identified in 441 (4.9%) of the cohort cases; 384 (87%) of these contained one RS, 42 (9.5%) contained two, nine (2%) contained 3, and six (1.5%) contained four or more, with a maximum of 11. RS size information was available in 434 cases. The majority of RS (357/434, 82%) were less than 5 mm in diameter; 61 biopsies (14%) contained 5-9.9 mm RS, and 16(4%) had RS than 10 mm. Table 1 and 2 compare the relative risk of BBD subsets defined by presence, size and number of RS to patient groups lacking RS.

**Conclusion:** RS imparts no increased breast cancer risk compared to other forms of PDWA (i.e. duct hyperplasia and/or adenosis). Likewise, RS associated with AH also connotes no increased risk above that of AH. Breast cancer risk was not modified significantly by the size or number of RS lesions.

Table 1: Incident Breast Cancer Relative Risk by Histology and RS Presence

Diagnosis	Number	Relative Risk (95% CI)
NP	6048	1.07 (0.96, 1.18)
PDWA	2311	1.57 (1.37, 1.80)
PDWA + RS	377	1.84 (1.33, 2.49)
AH	273	4.01 (3.03, 5.21)

AH + RS 64 3.33 (1.67, 5.97)

Relative risks were calculated using Cox Proportional Hazard Resgression Analysis. Results are adjusted for age.

Table 2: Relative Risk By Radial Scar Feature

Feature	Number	SIR (95% CI)
1 Scar	384	2.02 (1.48, 2.69)
2+ Scars	57	2.12 (0.85, 4.35)
Size < 5 mm	357	1.84 (1.32, 2.51)
Size > 5 mm	77	2.50 (1.20, 4.61)

Relative risks were calculated using Cox Proportional Hazard Regression Analysis. Results are adjusted for age.

Podium Presentation: American Association for Cancer Research, Annual Meeting, April 1-5, 2006, Washington, D.C.

**COX-2 expression in atypia: Correlation with breast cancer risk.**

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**Background:** Women with atypical hyperplasia have a significantly increased risk of a later breast cancer (RR~4.0) and are excellent candidates for chemoprevention strategies. Identification of appropriate molecular targets is a priority. COX-2 is up-regulated in a variety of malignancies by several oncogenic mechanisms. Increased COX-2 expression has been documented in DCIS specimens.<sup>1</sup> We sought to determine COX-2 expression in women with atypia and assess possible correlations with a later breast cancer.

**Methods:** The Mayo Clinic Benign Breast Disease Cohort includes 9343 women who had an open breast biopsy between 1967 and 1991.<sup>2</sup> For 247 women with atypical hyperplasia, there was formalin-fixed, paraffin-embedded tissue available for assessment of COX-2 expression by immunohistochemistry. Our study pathologist (DWV) scored the COX-2 expression on a scale from 0 (negative) to 3+ (high intensity). We used Cochran-Mantel-Haenszel tests for trend to compare intensity of staining in the samples of women who developed breast cancer (cases) to that of women who did not develop breast cancer (controls).

**Results:** Forty of the 247 women with atypia have developed breast cancer over a median follow-up of 15 years. The atypia samples displayed a range of COX-2 expression with values of 0 for 28 (11.3%), 1+ for 113 (45.8%), 2+ for 74 (30%), and 3+ staining for 32 (13%). We found significantly higher COX-2 staining intensity in the atypias of those women who went on to develop breast cancer compared to the controls who did not ( $p=0.04$ ).

**Conclusions:** Women with atypia are recognized as having a high risk for a later breast cancer. Intense COX-2 expression is associated with a significantly greater likelihood of a subsequent breast cancer in women with atypia and represents one potential molecular target for chemoprevention strategies.

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***Assessment of the Gail model in a cohort of women with atypical hyperplasia.***

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**Background:** Understanding an individual woman's risk of developing breast cancer is of high importance if we are to tailor clinical management properly. We sought to evaluate the performance of the Gail model<sup>1</sup> in a cohort of women with atypical hyperplasia, and to determine if other histopathological features might contribute to enhanced risk prediction in this cohort.

**Methods:** The Mayo Clinic Benign Breast Disease (BBD) Cohort includes 9343 women who had an open breast biopsy between 1967 and 1991.<sup>2</sup> Of these, 336 women had atypical hyperplasia, a group with significantly increased risk of a later breast cancer (RR~4.0). Gail model risk factors, and others, were obtained via survey and medical record review. Lifetime risk (thirty-year probability) of breast cancer was computed for each woman. Logistic regression was used to assess the concordance between the predicted and observed lifetime risk. Proportional hazards regression, with bootstrap model selection, was used to identify a potential risk prediction model for this high-risk group of women.

**Results:** In this atypia sub-cohort, 64 women experienced a breast cancer with an average follow-up of about 15 years. This number of events was slightly lower than the number predicted by the 30-year Gail model probabilities (rate ratio [95% CI] = 0.94 [0.74 - 1.20]). At the individual level, the concordance between observed breast cancer events and predicted lifetime probabilities of breast cancer was 0.59. This did not reach statistical significance (p=0.13), however, the number of events was low. The model selection process identified one covariate that was associated with breast cancer risk in this sub-cohort: the number of foci of atypia.

**Conclusions:** Averaging risks across this atypia cohort, the Gail model prediction was on target, but the per-individual concordance between observed and predicted breast cancer was low. Knowledge of the number of foci of atypia provided additional information about breast cancer risk. The development of alternative risk models in this group, and in the entire BBD cohort, are in process.

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**Epidemiologic Comparison of Disease Incidence Among Populations: The Person-Years Approach.**

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In epidemiological studies it is often of interest to compare disease incidence within a study cohort to that of a reference population. The person-years approach is often used to make and summarize such comparisons. The resulting Standardized Mortality Ratios (SMRs) summarize the degree to which observed cohorts differ from the reference population. While there have been criticisms of this method, there are few alternatives when one wishes to compare study groups with respect to their degree of deviation from population-based expectations. Our study of this topic is motivated by a desire to study the risks of breast cancer in women with a history of a benign breast biopsy relative to a reference population. In this study, follow-up data were obtained from a consecutive series of 9086 women having had a benign breast biopsy. Women with a history of a benign breast biopsy had an SMR of 1.6 (95% CI: 1.5 – 1.7). We present an overview of the person-years method and demonstrate how estimates of per-subject expected events may be used in the place of group-aggregated expected events. We also outline modifications that may alleviate concerns that arise in the use of this approach, motivated by data from the study that motivated our investigations.

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**Mammographic breast density is inversely associated with age-related involution.**

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**Background:** The breast epithelium is composed of acini that join to form lobules. These lobules undergo age-related involution that has been demonstrated in women even in their 30s. Mammographic breast density is a known risk factor for breast cancer that has also been shown to decrease with age. This study aims to examine the association of age-related lobular involution with mammographic breast density (MBD) in a cohort of women with benign breast disease (BBD).

**Materials and Methods:** Women from the Mayo BBD Cohort who were diagnosed with BBD between 1985 and 1991 and had a mammogram within 6 months of BBD diagnosis were eligible for this study. MBD as a 4-category measure of Bi-RADS density was ascertained from a clinical mammography database that has been maintained since 1985 at the Mayo Clinic. Our breast pathologist (DV), blinded to both the initial diagnosis and clinical outcome, performed pathology review of all the study tissue. Involution in the breast tissue was subjectively assessed as categories of none- 0% involution, partial- 1 – 74% involution, or complete- >75% involution of the terminal duct lobular units. To examine the association between involution and Bi-RADS density, the odds of a low density mammogram (Bi-Rads=1) were estimated for the three categories of involution using logistic regression.

**Results:** A total of 3773 women from the BBD cohort were diagnosed between 1985 and 1991; of these, 2667 (71%) had a Bi-RADS density available within 6 months of BBD diagnosis. Mean age at BBD diagnosis in this sample was 55 years. The distribution of involution and Bi-RADS category of density is shown in the table below.

Bi-RADS Breast density	Involution None	Involution Partial	Involution Complete	Total
1	56 (15.60%)	325 (19.51%)	174 (27.10%)	555 (20.81%)
2	31 (8.64%)	221 (13.27%)	126 (19.63%)	378 (14.17%)
3	54 (15.04%)	412 (24.73%)	176 (27.41%)	642 (24.07%)
4	218 (60.72%)	708 (42.50%)	166 (25.86%)	1092 (40.94%)
Total 359		1666	642	2667

Age adjusted analyses illustrate an inverse association of involution and Bi-RADS density. Compared to women with no evidence for involution, women with partial involution had 1.1 OR(CI 0.8,1.5) greater odds of low MBD and women with complete involution had 1.4 OR(CI 0.96,2.1) greater odds [p-value test for trend p=0.03].

**Discussion:** Our findings indicate that MBD is inversely associated with involution of breast tissues. Future histological and molecular studies are warranted to understand the process of age-related involution and its association with breast density, in order to improve our understanding of the biology of breast tissues, thereby, of breast cancer.

Poster Presentation: San Antonio Breast Cancer Symposium, Annual Meeting, December 16, 2006, San Antonio, TX.

Age-related lobular involution and reduced risk of breast cancer.

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**Background:** Lobular involution or regression in the human breast is a natural process associated with aging. To identify tissue-based markers of risk of breast cancer, we studied if the extent of lobular involution was associated with later risk of breast cancer.

**Methods:** We examined the benign breast biopsies of 8,736 women in the Mayo Benign Breast Disease Cohort and classified the lobules in the background breast tissue by degree of involution: none (0%), partial (1-74%), or complete ( $\geq 75\%$ ). Subsequent breast cancer events and epidemiologic data were obtained from medical records and questionnaires. For relative risks, we compared observed events to those expected, based on the Iowa Surveillance, Epidemiology, and End Results registry.

**Results:** Distribution by degree of involution was: none-1,627 (18.6%); partial-5,197 (59.5%); and complete-1,912 (21.9%). Increased involution correlated with increased age ( $p<0.0001$ ). Complete involution of lobular units was seen in 19/566 (3.4%) of women  $<30$  at benign biopsy; 53/1037 (5.1%) of those ages 30-39; 142/2446 (5.8%) of those ages 40-49; 455/2109 (21.6%) of those ages 50-59; 724/1600 (45.3%) of those ages 60-69; and 519/978 (53.1%) of those ages 70 and higher. There was a strong, inverse correlation ( $p<0.0001$ ) between involution and parity. Nulliparous women or women with only one child were more likely to have complete involution of their lobular units. Women with two, three, and four or more children had stepwise decrements in the likelihood of complete involution. The breast tissue from women with a strong family history was less likely to demonstrate involution ( $p=0.0006$ ).

After a median of 17 years, the relative risk of breast cancer in the cohort overall was 1.40 (95% CI 1.30-1.51). The risk of breast cancer varied significantly by extent of involution, ( $p<0.001$ ). For those women with no involution, the risk was 1.88 (95% CI 1.59-2.21); with partial involution, 1.47 (1.33-1.61); and with complete involution, 0.91 (0.75-1.10). Moreover, involution of lobular units modified the risk of breast cancer in all subsets, even women with atypia. Women with atypia and complete involution had a risk of 1.49 vs 4.06 with partial involution and 7.79 with no involution ( $p=0.003$ ). In women with a strong family history of breast cancer and no involution, the RR was 2.77 (95% CI 1.94-3.84), while those with complete involution had a RR of 1.61 (95% CI 0.92-2.61).

**Conclusions:** The degree of age-related lobular involution correlates inversely with breast cancer risk. Aberrant involution may be a biologically important variable in breast cancer biology that can better inform risk prediction at the time of breast biopsy.

Poster presentation: San Antonio Breast Cancer Symposium, Annual Meeting, December 16, 2006, San Antonio, TX.

**Centrosome Amplification is Greatest in Benign Breast Lesions Associated with an Increase in Risk of Cancer.**

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Although centrosome amplification is known to be present in invasive breast cancer and ductal carcinoma in situ, it has not been investigated in benign breast lesions. Benign breast disease (BBD) encompasses a spectrum of histologic entities, usually subdivided into non-proliferative lesions, proliferative lesions without atypia, and atypical hyperplasia. A modest increase risk in development of breast cancer in the future has been associated with proliferative lesions, whereas a significant increased risk is associated with atypical hyperplasias. In this pilot study we investigated the status of centrosomes in BBD of various histologies to determine if amplified centrosomes can be detected in the absence of malignancy and invasion, and if any histologic types of BBD have significant levels of centrosome amplification. This study utilizes tissues from patients with BBD who were seen at the Mayo Clinic between 1967 and 1991 and followed up for a median of 15 years. Paraffin embedded BBD sections were stained with hematoxylin and eosin (H&E) for histological assessment. Adjacent sections were immunostained for gamma tubulin as a marker of centrosomes. Images captured using an Apotome-equipped Zeiss Axiovert 200M or Zeiss 510 LSM microscope for z-sectioning. Maximum intensity projection images were analyzed for centrosome size and number. Fibroblast centrosomes, present in each image, were used for normalization. We performed a pilot analysis of 5 non-proliferative lesions, 5 proliferative lesions without atypia, and 42 with atypia. Centrosome amplification was present in 88% of atypical hyperplasia samples, compared to none of the non-proliferative lesions, and only 1 of the proliferative lesions without atypia. Analysis of the complete set of tissues (>40 of each type) will allow us to determine if this difference is significant, and if it is related to development of invasive breast cancer during the patient follow up period.

Poster Presentation: American Society of Clinical Oncology's Breast Cancer Symposium, September 2007, San Francisco, CA.

**Assessment of the accuracy of the Gail model in women with atypical hyperplasia.**

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**Background:** An accurate estimate of an individual woman's risk of breast cancer is essential for patient counseling and management. Women with atypical hyperplasia (AH) are at an elevated risk of breast cancer. Despite the widespread use of the Gail model, it has not been validated in patients with AH. We evaluate the accuracy of the Gail model in individuals from a well-annotated, well-characterized cohort of women with AH.

**Methods:** The Mayo Benign Breast Disease (BBD) Cohort is comprised of women aged 18 to 85 who had an open breast biopsy at the Mayo Clinic between 1967 and 1991 with benign pathological findings. Women with atypical ductal or lobular hyperplasia were identified by our study pathologists. Each individual's risk factors for breast cancer were obtained and the Gail model was used to predict five-year, follow-up specific and lifetime (age 90) risks for each woman. For the group level evaluation, the predicted numbers of breast cancers were compared to the observed numbers. For the individual-specific assessment, the concordance statistic was calculated.

**Results:** Of the 9,376 women in the cohort, 331 women (3.5%) had AH, which make up this study group. Over a mean follow up of 13.7 years, 66 (20%) women have developed breast cancer. The Gail model predicted 31.7 breast cancers in this length of follow up (rate ratio [95% CI] = 2.08 [1.64 – 2.65],  $p < 0.001$ ). The concordance statistic, which determines the individual-specific agreement between predicted and observed outcomes, suggested that the Gail model did not predict well at the individual level, with a concordance between the individual-specific risk predictions and the observed breast cancer events in the first five years of follow-up of 0.46 (95% CI: 0.27 – 0.64). When evaluated linked to length of follow-up the concordance was 0.58 (95% CI: 0.53 – 0.63).

**Conclusion:** The Gail model significantly underestimates the risk of breast cancer development in women with atypical hyperplasia. Physicians should be cautious when using the Gail model to counsel individual patients with atypical hyperplasia regarding their risk of breast cancer development.

Poster presentation: Society of Clinical Oncologists Breast Cancer Symposium, Washington, D.C., September 6, 2008.

**A novel, tissue-based feature strongly associated with breast cancer risk.**

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**Background:** Age-related involution of breast lobules (the anatomic substructure that gives rise to breast cancer), assessed qualitatively, is associated with reduced risk of breast cancer (Milanese et al, JNCI 2006). We hypothesized that a quantitative assessment of lobular involution could be developed to predict breast cancer risk more precisely.

**Methods:** We performed a nested case control study of 86 cases and 152 controls (238 patients) within the Mayo Clinic Benign Breast Disease Cohort. Hematoxylin and eosin stained slides were scanned into the computer and analyzed using Webslidebrowser software. The 10 largest normal lobules for each patient were analyzed for area measurement and number of acini per lobule. Means were compared for cases and controls, qualitative involution status (none, partial, or complete), histology (nonproliferative, proliferative disease without atypia, or atypical hyperplasia), and family history (none, weak, or strong).

**Results:** Women who went on to develop breast cancer had a larger lobular area ( $59,458\mu^2$  vs.  $49,221\mu^2$ ;  $p = 0.0452$ ) and higher number of acini per lobule (21.62 vs. 16.11;  $p = 0.0006$ ) than women who remained unaffected. Women with no involution had a larger lobular area ( $102,013\mu^2$ ) and number of acini per lobule (35.72) than women with partial ( $56,945\mu^2$ , 20.85 acini per lobule) or complete involution ( $27,254\mu^2$ , 8.72 acini per lobule) ( $p < 0.0001$ ). The difference between lobular area and number of acini per lobule was not statistically significant when evaluating for effect of histology or family history ( $p = 0.153$  and  $0.4770$ , respectively).

**Conclusions:** Lobular involution can be quantified and may be useful as a risk predictor for women who have had benign breast biopsies.

Podium Presentation: San Antonio Breast Cancer Symposium, December 10-14, 2008, San Antonio, TX.

**Benign breast disease and breast cancer risk in young women.**

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**Background:** Breast cancer is the leading cause of cancer deaths in younger women (25 to 49 years of age). Young women with breast cancer also have worse overall survival and increased risk of recurrence compared to older women with breast cancer. Innovative approaches to understanding risk factors and tissue characteristics for the younger population can improve understanding of breast cancer etiology and enhance risk-stratification for these women. This study was aimed at examining breast cancer risk factors among young women (<50 years) with BBD.

**Materials and Methods:** Utilizing the Mayo Clinic Surgical and Pathology Indices, women ages 18 to 85 who had benign excisional breast biopsy between January 1, 1967 and December 31, 1991 were identified. The diagnosis of breast cancer served as the study endpoint and was determined using the Mayo medical record and questionnaire information from study participants. The breast pathologist, blinded to the initial diagnosis and clinical outcome, performed pathology review. BBD was classified as non-proliferative disease (NPD), proliferative disease without atypia (PDWA), or atypical hyperplasia (AH). Age-related lobular involution (reduction in number and size of acini per lobule) was classified as none-0%, partial- 1 to 74%, or complete-  $\geq$ 75% involution. Relative risk (RR) was estimated by comparing the number of observed breast cancers with the number expected, based on breast cancer rates in the Iowa Surveillance, Epidemiology, and End Results registry.

**Results:** Of the 9376 women in the Mayo BBD cohort, 4460 women were aged <50 years at BBD diagnosis and formed the study cohort. The mean age at BBD diagnosis was 39.4 ( $\pm$  8.3) years. With a median follow-up of 20 years, 326 breast cancer cases were identified. The histologic findings were NPD in 72% of women, PDWA in 26%, and AH in 2%. The relative risk of breast cancer for the overall cohort of young women with BBD was 1.5 (95% CI [1.4, 1.7]). The relative risk among those with AH was 6.9 (95% CI [4.6, 10.1]), compared with a RR of 2.0 (95% CI [1.7, 2.4]) for PDWA, and RR of 1.2 (95% CI [1.0, 1.4]) for NPD. Risk was associated with extent of lobular involution (RR for no involution was 1.7 (95%CI [1.4, 2.1]); partial involution 1.4 (95%CI [1.2, 1.7]); complete involution 0.7 (95%CI [0.3, 1.4])). Family history was available for 83% of the cohort and RR was 2.2 (95% CI [1.7, 2.8]) for women with strong family history and was 1.3 (95% CI [1.1, 1.6]) for women with no family history.

**Discussion:** Young women with BBD are at increased risk of breast cancer. Risk is high in women with atypical hyperplasia, and those with a family history of breast cancer. Lobular involution is associated with reduced breast cancer risk in this population, suggesting a role in modifying breast cancer risk. These findings suggest the need for further research in this population, along with tissue-based studies to examine the processes leading to breast cancer, and enable identification of those women at highest risk.

**Estrogen Receptor Expression in Atypical Hyperplasia and Its Association with Type of Atypia and Age.**

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**Background:** Estrogen receptor (ER) expression is present in normal breast epithelium and premalignant breast lesions. Prior studies have shown that ER expression increases with age in normal breast epithelium; whereas no age association was seen in atypical hyperplasia and carcinoma *in situ*.

**Design:** ER expression was assessed immunohistochemically in archival sections from 246 women with atypical hyperplasia who had an open benign breast biopsy between 1967 and 1991. The ACIS®III (Dako, Carpinteria, CA) was utilized to calculate ER expression (percent staining and staining intensity) in all atypical foci for each woman. Using multivariate linear regression, we examined associations of ER expression with age at biopsy, year of biopsy, indication for biopsy, type of atypia, number of atypical foci, involution status, and family history. Heterogeneity of breast cancer risk across levels of ER expression was also assessed, standardized to a control population (the Iowa SEER registry).

**Results:** Among the 246 women, 87 (35%) had ADH, 141 (57%) had ALH, and the remaining 18 (7%) had both ADH and ALH. About half (53%) were older than 55 years at diagnosis. Forty-nine (20%) developed breast cancer during a median follow up of 14.4 years. Multivariate analysis indicated that type of atypia, year of biopsy, and age at diagnosis were significant predictors of ER percent staining and intensity [ $P<0.05$  (see Table 1)]. ER expression was increased in women with ADH and/or those over the age of 55. The relationship between ER (percent staining and intensity) and breast cancer risk in patients diagnosed with atypia was not significant ( $P=0.099$  and  $P=0.118$ , respectively).

Poster Presentation: United States and Canadian Academy of Pathology, Annual Meeting, March 7-13, 2009, Boston, MA.

**Benign Breast Disease in African-American Women.**

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**Background:** Women with benign breast disease (BBD) represent a large and clinically important population. Specific histologic findings in BBD have been shown to be strong indicators of later risk of breast cancer. The major studies of BBD performed to date have been based primarily in Caucasian-American (CA) women. Thus, the prevalence and distribution pattern of BBD in African American (AA) women is not well known.

**Design:** We reviewed archival H and E stained sections of breast needle core and excisional biopsies performed on all AA women in the years 1998 to 2000 at our institution and diagnosed with BBD. BBD was classified, by one pathologist, as nonproliferative (NP), proliferative disease without atypia (PDWA) or atypical hyperplasia including ductal and lobular types (AH), using standard microscopic criteria. We also examined the status of lobular involution in the same biopsies and classified it as none or absent (<1%), partial (1-75%), or complete (75%). We compared lobular involution in our population to that of a cohort of CA women with a diagnosis of BBD within the same age category (<45y; 45-55y and >55y).

**Results:** We identified 520 AA patients with a diagnosis of BBD on breast biopsy. The mean age at diagnosis was 46.4 years (14.7 y). Seventy-five percent were diagnosed with NP, 22% with PDWA, and 3% with AH. Lobular involution increased with age. In women older than 55 years however, the increase in lobular involution appeared to be slower in AA than in CA women (CA had 2.7% none vs. 44% complete and AA had 19% none vs. 31% complete;  $p<0.001$ ). There was no difference between AA and CA women younger than 55 years in regard to the presence of lobular involution. Lobular involution was similar throughout the different BBD categories in the AA population.

**Conclusions:** In our series of AA women with BBD, the distribution of BBD appears to be similar to other series (including mainly CA women). Our data on lobular involution may indicate that AA undergo lobular involution at a different rate than CA women.

Poster Presentation: United States and Canadian Academy of Pathology, Annual Meeting, March 7-13, 2009, Boston, MA.

**Clinical Analysis of Mucocele-Like Tumors of the Breast: Analysis of a Large Benign Breast Disease Cohort.**

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**Background:** Mucocele-like tumors (MLT) of the breast are unusual lesions characterized by cysts distended with mucin that also dissects/extravasates through the epithelium into surrounding stroma. They are accompanied by variable epithelial proliferation, with an increased frequency of associated atypical ductal hyperplasia (ADH). It is not known whether MLT represent a risk factor for subsequent development of breast carcinoma.

**Design:** Our benign breast disease cohort is comprised of 9376 women who underwent excisional breast biopsy from 1967-1991. Slides from all patients were reviewed retrospectively in a blinded fashion and classified per standard diagnostic criteria by two study pathologists. Mean follow up is 13.7 years. We analyzed subjects with MLT diagnoses for the frequency of proliferative lesions, including ALH/ADH, and for their likelihood of developing breast cancer.

**Results:** The cohort contained 70 MLT (0.75%). Thirty patients (42.9%) were >55 yrs of age at time of diagnosis, 24 (34.3%) were 45-54 yrs and 16 (22.9%) were <45 yrs. MLT were more often associated with proliferative lesions (70% in MLT vs 33% for the cohort overall). ALH/ADH was present in 21.4% MLT lesions, vs 3.4% in the cohort overall ( $p<0.0001$ ). To date, 6/70 patients with MLT (8.6%) have developed breast carcinoma; this frequency is not significantly different than the BBD cohort overall ( $p=0.8780$ ).

**Conclusions:** Our findings support previous studies showing a relationship between MLT and atypia. However, beyond the risk associated with atypia itself, we do not observe an additional risk of breast carcinoma associated with the presence of the MLT.

## **Appendix C: Manuscripts**

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## Benign Breast Disease and the Risk of Breast Cancer

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### ABSTRACT

#### BACKGROUND

Benign breast disease is an important risk factor for breast cancer. We studied a large group of women with benign breast disease to obtain reliable estimates of this risk.

#### METHODS

We identified all women who received a diagnosis of benign breast disease at the Mayo Clinic between 1967 and 1991. Breast-cancer events were obtained from medical records and questionnaires. To estimate relative risks, we compared the number of observed breast cancers with the number expected on the basis of the rates of breast cancer in the Iowa Surveillance, Epidemiology, and End Results registry.

#### RESULTS

We followed 9087 women for a median of 15 years. The histologic findings were non-proliferative lesions in 67 percent of women, proliferative lesions without atypia in 30 percent, and atypical hyperplasia in 4 percent. To date, 707 breast cancers have developed. The relative risk of breast cancer for the cohort was 1.56 (95 percent confidence interval, 1.45 to 1.68), and this increased risk persisted for at least 25 years after biopsy. The relative risk associated with atypia was 4.24 (95 percent confidence interval, 3.26 to 5.41), as compared with a relative risk of 1.88 (95 percent confidence interval, 1.66 to 2.12) for proliferative changes without atypia and of 1.27 (95 percent confidence interval, 1.15 to 1.41) for nonproliferative lesions. The strength of the family history of breast cancer, available for 4808 women, was a risk factor that was independent of histologic findings. No increased risk was found among women with no family history and nonproliferative findings. In the first 10 years after the initial biopsy, an excess of cancers occurred in the same breast, especially in women with atypia.

#### CONCLUSIONS

Risk factors for breast cancer after the diagnosis of benign breast disease include the histologic classification of a benign breast lesion and a family history of breast cancer.

From the Divisions of Medical Oncology (L.C.H., M.H.F., J.J.), Experimental Pathology (W.L.L.), General Surgery (A.C.D.), General Internal Medicine (K.G.), Biostatistics (R.A.V., S.D.M., V.S.P., D.W.H., V.J.S.), Epidemiology (C.M.V., L.J.M.), and Anatomic Pathology (D.W.V.), Mayo Clinic College of Medicine, Rochester, Minn.; H. Lee Moffit Cancer Center and Research Institute, Tampa, Fla. (T.A.S.); Wayne State University, Detroit (C.R.); and the University of California, San Francisco, San Francisco (T.T.). Address reprint requests to Dr. Hartmann at Mayo Clinic College of Medicine, Rochester, MN 55905.

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**B**ENIGN BREAST DISEASE IS AN IMPORTANT risk factor for a later breast cancer, which can develop in either breast.<sup>1</sup> It encompasses a spectrum of histologic entities, usually subdivided into nonproliferative lesions, proliferative lesions without atypia, and atypical hyperplasias, with an increased risk of breast cancer associated with proliferative or atypical lesions.<sup>2-4</sup> The identification of benign breast disease has become more common as the use of mammography has increased, and thus, having accurate risk estimates for women who receive this diagnosis is imperative.

Important questions remain, however, about the degree of risk associated with the common nonproliferative benign entities and the extent to which family history influences the risk of breast cancer in women with proliferative or atypical lesions. Dupont and Page found that women with nonproliferative disease did not have an increased risk of a later breast cancer.<sup>2</sup> By contrast, a companion study to the National Surgical Adjuvant Breast and Bowel Project (NSABP) Breast Cancer Prevention Trial (P1) found a relative risk of 1.6 for women who received a diagnosis of a "lower category" of benign breast disease.<sup>5</sup> A limitation of the NSABP study however, was the lack of central pathological review.

Another major question concerns the possible interplay between atypia and a family history of breast cancer. The Dupont and Page study found that women with atypia and a family history had 11 times the risk of those with nonproliferative lesions and no family history.<sup>2</sup> However, two other major studies of benign breast disease<sup>6,7</sup> did not find a significant interaction between atypia and family history. The duration of increased risk after a finding of benign disease on biopsy is also uncertain.<sup>2,4,8</sup>

Studies of benign breast disease can also clarify whether there is a continuum of breast alterations that culminates in breast cancer. However, it remains unclear which of the benign entities are actual precursors and which reflect a background of increased risk involving all breast tissue in a woman. Determining the extent of agreement between the side (right or left) of the benign lesion and the subsequent breast cancer is one means of assessing these issues.

To investigate these questions, we studied 9087 women with benign breast disease for whom we had follow-up data on breast-cancer events. This cohort has been followed for a median of 15 years, and 707 breast cancers have developed, making this, to our knowledge, one of the largest such studies of its

kind. We report on the risk of breast cancer according to histologic findings, the age at diagnosis of benign breast disease, and the strength of the family history. We also recorded the side of the cancer (ipsilateral or contralateral) and the time to the diagnosis of cancer.

## METHODS

### STUDY POPULATION

We accessed data from the Mayo Clinic Surgical Index and Pathology Index to identify all women 18 to 85 years of age who had undergone surgical excision of a benign breast lesion during the 25-year period from January 1, 1967, through December 31, 1991. For women who had more than one biopsy during this period, we used the first sample. The original list contained 12,132 women, but we excluded 1,047 women for any of the following: a diagnosis of breast cancer or lobular carcinoma in situ at, before, or within six months after the biopsy of the benign lesion; mastectomy (unilateral or bilateral) or breast reduction at or before biopsy; or refusal to allow use of their medical records for research.<sup>9</sup> This left 11,085 women. Of these, 10,531 (9.5 percent) had no follow-up information after the biopsy. Thus, a total of 10,032 women met our criteria for study entry and had follow-up information. Of these, 945 women had unusable or unavailable biopsy specimens of the benign lesion. The remaining group of 9087 women constitutes our study cohort. The relative risks of breast cancer (described below) did not differ significantly between the 10,032 women who met our criteria and the 9087 women who made up the study cohort (1.59 and 1.56, respectively).

### FAMILY HISTORY AND FOLLOW-UP

A questionnaire designed for this study was used to obtain information about family history and other possible risk factors for breast cancer. Thus, our family-history data were obtained at the time of follow-up contact. We categorized family history as none, weak, or strong. The criteria for a strong family history were as follows: at least one first-degree relative with breast cancer before the age of 50 years or two or more relatives with breast cancer, with at least one being a first-degree relative. Any lesser degree of family history of breast cancer was categorized as weak. The questionnaire also asked about breast-cancer occurrences. Follow-up for breast-cancer events was also obtained through the comprehensive (inpatient and outpatient) Mayo medical

record. Questionnaire information was available for 5619 women (61.8 percent). Of the questionnaires, 604 (10.7 percent) were completed by proxy (the next of kin of a deceased patient). As of August 1, 2004, 7260 (79.9 percent) members of the cohort were still alive. All protocol procedures and patient-contact materials were reviewed and approved by the institutional review board of the Mayo Clinic; returning the contact materials was considered implied consent.

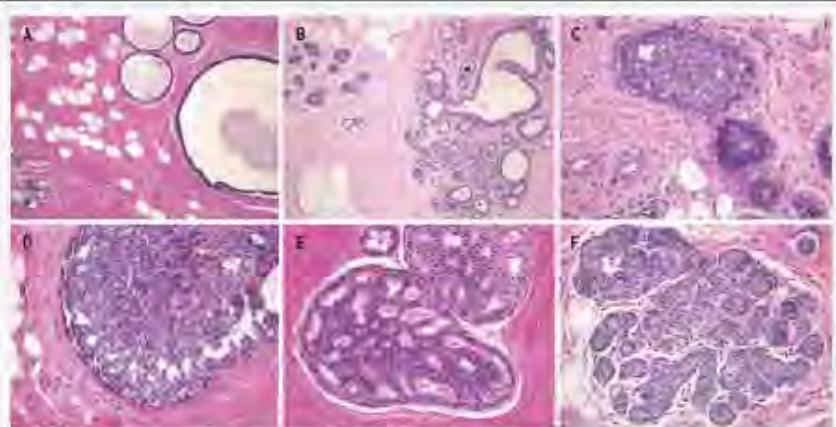
#### HISTOLOGY

Stored hematoxylin-and-eosin-stained sections from each participant were evaluated by a breast pathologist who was unaware of the initial histologic diagnoses and patient outcomes. Biopsy findings were classified according to the criteria of Page et al.<sup>2,10</sup> into the following categories: nonproliferative fibrocystic changes, proliferative fibrocystic changes without atypia, and proliferative fibrocystic changes

with atypia (atypical ductal hyperplasia, atypical lobular hyperplasia, or both) (Fig. 1).<sup>2,10</sup> Biopsy specimens were designated as having proliferative fibrocystic changes if they contained any of the following: ductal hyperplasia (greater than mild), papilloma, radial scar, or sclerosing adenosis. Cysts, fibroadenoma, or columnar changes were considered nonproliferative unless they also contained one of the lesions denoted above.

#### STATISTICAL ANALYSIS

The duration of follow-up was calculated as the number of days from biopsy of the benign lesion to the date of the diagnosis of breast cancer, death, or last contact. We estimated relative risks on the basis of standardized incidence ratios (SIRs), dividing the observed numbers of incident breast cancers by population-based expected counts. We calculated these expected counts by apportioning each woman's follow-up into five-year age and calendar



**Figure 1.** Histopathological Appearance of Benign Breast Disease (Hematoxylin and Eosin).

Panel A shows nonproliferative fibrocystic changes: the architecture of the terminal-duct lobular unit is distorted by the formation of microcysts, associated with interlobular fibrosis. Panel B shows proliferative hyperplasia without atypia. This is adenosis, a distinctive form of hyperplasia characterized by the proliferation of lobular acini, forming crowded gland-like structures. For comparison, a normal lobule is on the left side. Panel C also shows proliferative hyperplasia without atypia. This is moderate ductal hyperplasia, which is characterized by a duct that is partially distended by hyperplastic epithelium within the lumen. Panel D again shows proliferative hyperplasia without atypia, but this is florid ductal hyperplasia: the involved duct is greatly expanded by a crowded, jumbled-appearing epithelial proliferation. Panel E shows atypical ductal hyperplasia: these proliferations are characterized by a combination of architectural complexity with partially formed secondary lumens and mild nuclear hyperchromasia in the epithelial-cell population. Panel F shows atypical lobular hyperplasia: monotonous cells fill the lumens of partially distended acini in this terminal-duct lobular unit.

period categories, thereby accounting for differences associated with these variables. We used the Iowa Surveillance, Epidemiology, and End Results (SEER) registry as the reference population because of its demographic similarities to the Mayo Clinic population (80 percent of cohort members reside in the upper Midwest). Over 95 percent of our cohort was white, equivalent to that reported in Iowa census data during the study period.<sup>11</sup> In the SIR analyses, we considered the time since the original biopsy as a time-dependent variable and all other factors as fixed.

Associations between the risk of breast cancer and histologic findings, the age at diagnosis of be-

nign breast disease, and the strength of the family history of cancer, as well as pairwise combinations of these variables, were examined with the use of Cox proportional-hazards regression analysis. The main effects for each categorized variable and the corresponding interaction terms were included in each model, and the statistical significance of each interaction was evaluated with the use of a multiple-degree-of-freedom likelihood-ratio test.

We studied ipsilateral and contralateral breast cancer as a function of the time since biopsy by estimating the relative risk of cancer in the same as compared with the opposite breast for five-year intervals. When calculating the incidence of ipsilateral

**Table 1.** Characteristics of the Women According to the Histologic Category of Benign Breast Disease.<sup>a</sup>

Characteristic	All Women (N=9087)	Nonproliferative Disease (N=6061)	Proliferative Disease without Atypia (N=2600)	Atypical Hyperplasia (N=316)
Percentage of total	100.0	66.7	29.6	3.7
Age at biopsy — no. of women (%)				
<40 yr	1841 (20.3)	1500 (24.7)	323 (12.0)	14 (5.4)
40–49 yr	2474 (27.2)	1621 (26.7)	770 (28.6)	83 (24.7)
50–59 yr	2145 (23.6)	1297 (21.4)	759 (28.2)	89 (26.5)
60–69 yr	1639 (18.0)	1014 (17.1)	522 (19.4)	81 (24.7)
≥70 yr	988 (10.9)	609 (10.0)	316 (11.7)	61 (18.8)
Mean age at biopsy —yr	51.4±14.3	49.9±14.8	53.9±12.6	57.8±12.3
Menopausal status at biopsy — no. of women (%) <sup>†</sup>				
Premenopausal (<45 yr)	2948 (32.4)	2246 (37.1)	652 (24.2)	50 (14.9)
Perimenopausal (45–55 yr)	2583 (28.4)	1610 (26.6)	871 (32.4)	102 (30.4)
Postmenopausal (>55 yr)	3556 (39.1)	2205 (36.4)	1167 (43.4)	184 (54.8)
Family history of breast cancer — no. of women (%)				
Unknown	4279 (47.1)	2970 (49.0)	1170 (43.5)	139 (41.4)
Known	4808 (52.9)	3091 (51.0)	1520 (56.5)	107 (31.6)
None	2668 (55.5)	1755 (56.1)	831 (54.7)	102 (51.8)
Weak	1174 (24.4)	736 (24.5)	378 (24.9)	46 (20.3)
Strong	966 (20.1)	600 (19.4)	311 (20.5)	55 (27.9)
Breast-cancer status as of August 2004 — no. of women (%)				
Negative	8380 (92.2)	5662 (93.7)	2426 (90.2)	272 (81.0)
Positive	707 (7.8)	379 (6.3)	264 (9.8)	64 (19.0)
Vital status — no. of women (%)				
Deceased	1827 (20.1)	1172 (19.3)	566 (21.0)	89 (26.5)
Alive	7260 (79.9)	4889 (80.7)	2124 (79.0)	247 (73.5)

\* Plus-minus values are means ± SD.

† Menopausal status was categorized according to the age at breast biopsy.

eral cancer, we censored follow-up on women with contralateral cancer after the date of diagnosis. Similarly, when calculating the incidence of contralateral cancer, we censored follow-up on women with ipsilateral cancer after the date of diagnosis. Data on women missing information on the side of the cancer or women who had bilateral biopsies or cancer were not included in these analyses. This approach yields identical numbers of person-years for each type of event. As a result, the length of follow-up is no longer a factor in the analysis and the relative risks are equivalent to simple ratios of event counts. We therefore used properties of the binomial distribution to obtain exact P values and 95 percent confidence intervals for these relative risks.<sup>12</sup> Statistical tests were two-sided, and analyses were conducted with the use of SAS (SAS) and Splus (Insightful) software.

## RESULTS

## CHARACTERISTICS OF PATIENTS AND PATHOLOGICAL SPECIMENS

The final cohort consisted of 9087 women with benign breast disease as determined by open surgical biopsy. Table 1 shows the age at the time of the biopsy, likely menopausal status on the basis of age, and the strength of the family history of breast cancer according to the histologic findings for the benign lesion. The broad histologic classifications included nonproliferative disease in 6061 (66.7 percent), proliferative disease without atypia in 2690 (29.6 percent), and atypical hyperplasia in 336 (3.7 percent). Figure 1 shows examples of these lesions. The mean age was 51.4 years, but women with nonproliferative findings were slightly younger, whereas those with atypia tended to be older (mean age,

Table 2. Risk Factors for Breast Cancer after the Diagnosis of Benign Breast Disease.<sup>a</sup>

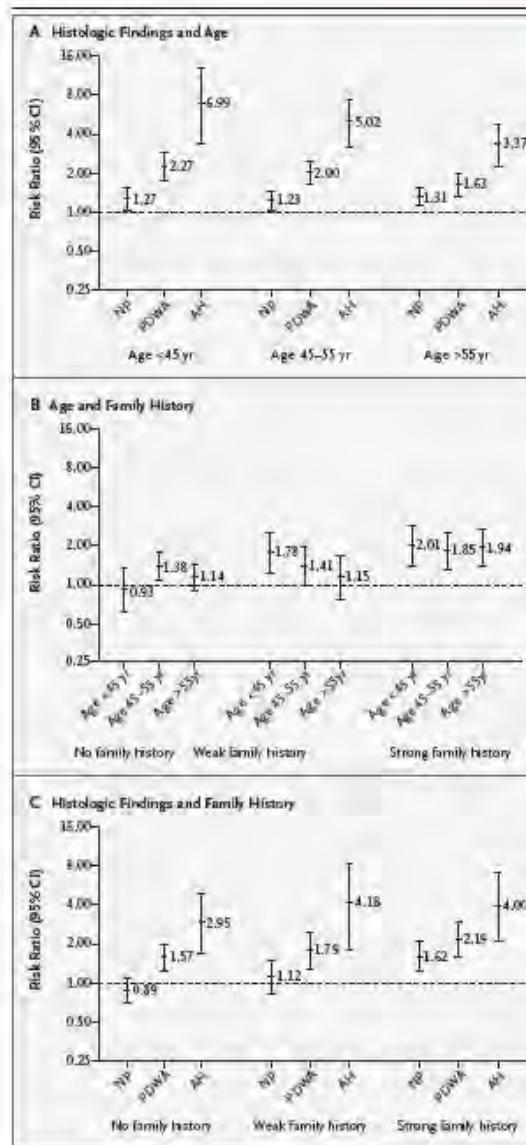
Characteristic	No. of Women	Person-Years	No. of Observed Events	No. of Expected Events	Relative Risk (95% CI) <sup>†</sup>
Overall	9087	144,881	707	453.0	1.56 (1.45–1.68)
Age at diagnosis of benign breast disease					
<30 yr	726	13,593	21	11.5	1.83 (1.13–2.80)
30–39 yr	1115	20,169	71	38.3	1.85 (1.45–2.34)
40–49 yr	2474	45,780	212	136.3	1.56 (1.35–1.78)
50–59 yr	2145	34,100	196	125.9	1.56 (1.35–1.79)
60–69 yr	1639	21,364	142	94.5	1.50 (1.27–1.77)
≥70 yr	988	9,874	65	46.6	1.40 (1.08–1.78)
Menopausal status <sup>‡</sup>					
Premenopausal (age <45 yr)	2948	54,419	169	106.1	1.59 (1.36–1.85)
Perimenopausal (age 45–55 yr)	2583	45,872	245	153.4	1.60 (1.40–1.81)
Postmenopausal (age >55 yr)	3556	44,590	293	198.6	1.51 (1.35–1.70)
Histologic findings					
Nonproliferative disease	6061	99,109	379	297.7	1.27 (1.15–1.41)
Proliferative disease without atypia	2690	41,610	264	140.2	1.88 (1.66–2.12)
Atypical hyperplasia	336	4,161	64	15.1	4.24 (3.26–5.41)
Family history of breast cancer <sup>§</sup>					
None	2668	44,974	171	145.4	1.18 (1.01–1.37)
Weak	1174	21,472	94	65.9	1.43 (1.15–1.75)
Strong	966	18,087	116	57.0	1.99 (1.58–2.32)

<sup>a</sup> Numbers of women, person-years, and events may not sum to overall totals because of rounding.

<sup>†</sup> The relative risk reflects the observed number of events as compared with the number expected on the basis of Iowa SEER data. All analyses account for the effects of age and calendar period. CI denotes confidence interval.

<sup>‡</sup> Menopausal status was categorized according to the age at breast biopsy.

<sup>§</sup> Information on family history was available for 4808 of the 9087 women.



**Figure 2.** Risk-Factor Interaction Profiles for Benign Breast Disease, Comparing the Number of Events Observed with the Number Expected.

Expected events account for age and calendar period and are calculated with the use of Iowa SEER rates. CI denotes confidence interval; NP, nonproliferative disease; PDWA, proliferative disease without atypia; and AH, atypical hyperplasia.

weakly positive in 1174 (24.4 percent), and strongly positive in 966 (20.1 percent). More women with atypia than without atypia had a strong family history of breast cancer (27.9 percent vs. 19.8 percent,  $P=0.06$ ). The risk of cancer was highest in the group with atypia: breast cancer developed in 64 of the 336 women (19.0 percent).

#### FEATURES OF BENIGN BREAST DISEASE AND SUBSEQUENT RISK OF BREAST CANCER

Patients in the cohort were followed for a median of 15 years. A total of 1827 women (20.1 percent) had died and 7260 (79.9 percent) were alive as of August 2004. We have documented 707 breast cancers to date. The median time from the original biopsy to the diagnosis of breast cancer was 10.7 years. Table 2 shows the estimated relative risks of breast cancer associated with the age at the initial biopsy, the strength of the family history, menopausal status, and histologic findings of the biopsy, as compared with expected population-based incidence. The estimated relative risk of breast cancer in the cohort was 1.56 (95 percent confidence interval, 1.45 to 1.68). The risk was inversely associated with the age at biopsy, with younger women having a greater risk than older women. The type of benign breast disease identified at biopsy was a major predictor of risk. Atypical hyperplasia had a relative risk of 4.24 (95 percent confidence interval, 3.26 to 5.41), proliferative disease without atypia had a relative risk of 1.88 (95 percent confidence interval, 1.66 to 2.12), and nonproliferative lesions had a relative risk of 1.27 (95 percent confidence interval, 1.15 to 1.41). Family history was an independent risk factor. For women with no known family history of breast cancer, the relative risk was only 1.18 (95 percent confidence interval, 1.01 to 1.37), as compared with 1.43 (95 percent confidence interval, 1.15 to 1.75) for women with a weak family history and 1.93 (95 percent confidence interval, 1.58 to 2.32) for those with a strong family history.

49.9 and 57.8 years, respectively;  $P<0.001$ ). Information on family history was available for 4808 women and was negative in 2668 (55.5 percent),

Figure 2 shows possible interactions between pairs of the major risk factors of age, histologic findings, and family history. No significant interactions were observed between age and family history or between histologic findings and family history, including atypia and family history. However, there was a significant interaction between age and histologic findings ( $P=0.05$ ): the risk of breast cancer was 6.99 times the expected risk among women who received a diagnosis of atypia before the age of 45 years; the risk was 5.02 times the expected risk when the atypia was diagnosed between the ages of 45 and 55 years and 3.37 times the expected risk when it was diagnosed after the age of 55 years. An important finding was that for women with nonproliferative disease and no family history or a weak family history, there was no increase in the risk of breast cancer.

#### TIME COURSE AND SIDE OF BREAST CANCER AFTER BENIGN BREAST DISEASE

Figure 3 shows the observed and expected numbers of cancers at five-year intervals. The excess risk persisted for at least 25 years after the initial biopsy and perhaps for 30 years or more, but accuracy was low after 25 years. Figure 4 shows a further breakdown of breast cancers into ipsilateral or contralateral according to the histologic findings in the benign lesion. Of the 616 unilateral cancers, 342 (55.5 percent) developed in the same breast as the initial biopsy and 274 (44.5 percent) developed in the contralateral breast. In the remaining 91 cases, there were bilateral events, either benign or malignant, or information on the side of the cancer was missing. During the first 10 years, there was an excess of ipsilateral cancers, with relative risks of ipsilateral as compared with contralateral cancer of 1.88 (95 percent confidence interval, 1.33 to 2.64) for years 0 through 5 and 1.34 (95 percent confidence interval, 0.96 to 1.85) for years 6 through 10. The 35 women with atypia in whom breast cancer developed within 10 years after the initial biopsy were 2.5 times as likely ( $P=0.02$ ) to have the cancer in the same breast as in the opposite breast.

#### DISCUSSION

Retrospective and prospective studies have shown a relative risk of breast cancer of 1.5 to 1.6 for women with benign breast disease as compared with women in the general population.<sup>2,5-7,19-21</sup> The histologic appearance of the benign lesion is a major

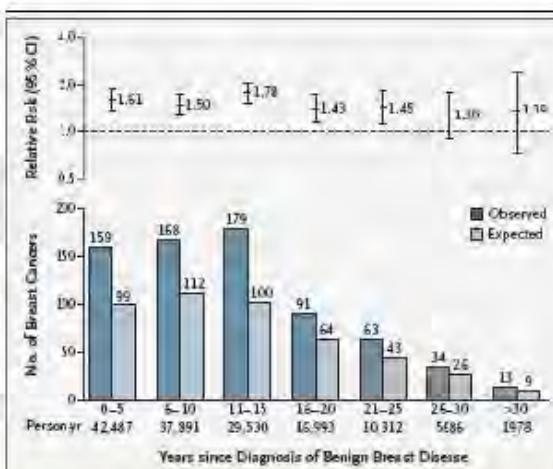
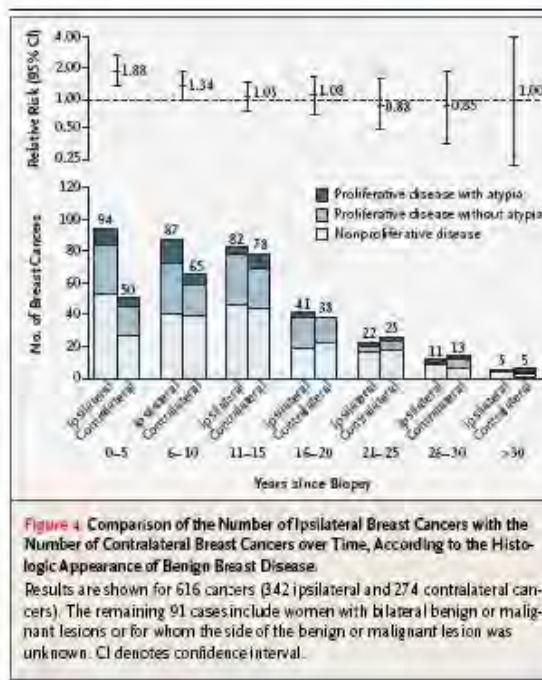


Figure 3. The Number of Breast Cancers Observed as Compared with the Number Expected over Time.

Expected events account for age and calendar period and are calculated with the use of Iowa SEER rates. CI denotes confidence interval.

determinant of risk, yet not all large studies have had access to tissue for re-review. Our investigation was based on a single institution resource with long-term and complete follow-up for cancer events. All samples containing the benign lesion were read by a breast pathologist who applied current histologic classifications. More than 700 breast cancers developed in this cohort, giving our study good statistical power. The relative risk of breast cancer for our cohort overall was 1.56 (95 percent confidence interval, 1.45 to 1.68), and this increased risk persisted for at least 25 years after the initial biopsy.

The histologic appearance of the benign lesion is strongly associated with the risk of breast cancer. For biopsies with nonproliferative findings, the relative risk was 1.27 (95 percent confidence interval, 1.15 to 1.41), as compared with a relative risk of 1.88 (95 percent confidence interval, 1.66 to 2.12) for findings of proliferative changes but no atypia and of 2.4 (95 percent confidence interval, 3.26 to 5.41) for a finding of atypical hyperplasia. When the family history is known, risk profiles can be refined. For women with nonproliferative findings and no family history or a weak family history of breast cancer, we observed no increased risk. This finding is important, because a sizable proportion



**Figure 4.** Comparison of the Number of Ipsilateral Breast Cancers with the Number of Contralateral Breast Cancers over Time, According to the Histologic Appearance of Benign Breast Disease. Results are shown for 616 cancers (342 ipsilateral and 274 contralateral cancers). The remaining 91 cases include women with bilateral benign or malignant lesions or for whom the side of the benign or malignant lesion was unknown. CI denotes confidence interval.

of women with benign breast disease are in this group (52 percent of our cohort with a known family history status). Dupont and Page made a similar observation in their 1985 report.<sup>21</sup> However, a recent NSABP study found a significantly increased risk of breast cancer among women with lower-category benign breast disease, including nonproliferative disease.<sup>5</sup> In the NSABP P1 trial, which included more than 13,000 women, 1376 had a breast biopsy with benign findings over a mean follow-up period of 79 months. Breast cancer developed in 47 of these women. On the basis of pathology reports from contributing centers, the investigators reported a relative risk of 1.6 among women with lower category findings on breast biopsy as compared with P1 participants who did not undergo a breast biopsy.<sup>5</sup>

In our study, the degree of family history was an independent risk factor. In women with a strong family history of breast cancer, even nonproliferative findings were associated with a risk ratio of 1.62. This subgroup may parallel the high-risk NSABP cohort.<sup>5</sup> Women with atypia are at significantly in-

creased risk, but a family history did not significantly modify the atypia-associated risk (Fig. 2). The risk was four times the expected risk among women with atypia and a family history of breast cancer, regardless of the degree of their family history; among women with atypia without a family history of breast cancer, the risk ratio was 2.95 (95 percent confidence interval, 1.65 to 4.87).

The age at the diagnosis of benign breast disease appears to modify the risks related to the histologic appearance of benign breast disease. The presence of atypia in women under 45 years of age conveyed twice the risk observed among women over 55 years of age (6.99 and 3.37, respectively), which might relate, in part, to menopausal status. The Breast Cancer Detection and Demonstration Project showed that the risk of breast cancer among premenopausal women with atypia was elevated by a factor of 12.0 (95 percent confidence interval, 2.0 to 68.0), as compared with 3.3 among postmenopausal women with atypia (95 percent confidence interval, 1.1 to 10.0), but the numbers of patients in the study were small.<sup>22</sup> The Nurses Health Study also showed an increased risk of breast cancer among premenopausal women with atypia.<sup>7</sup> However, in the NSABP study of women with lower categories of benign breast disease, the risk of breast cancer was greatest among postmenopausal women.<sup>5</sup>

Understanding the risk associated with benign breast disease is important because the increasing use of mammography has increased the frequency of breast biopsies, most of which yield benign findings. In a retrospective study of women undergoing annual mammographic screening, Elmore et al, found that 18.6 percent of women underwent a biopsy after 10 screening mammograms.<sup>23</sup> The use of hormone therapy may also affect the frequency of breast biopsies. Chlebowski et al., reporting for the Women's Health Initiative investigators, found that relatively short-term therapy with estrogen plus progestin increased the percentage of women with abnormal mammograms, a major indicator for breast biopsy.<sup>24</sup>

Regarding the possibility of malignant precursors within benign breast disease, we have information on the side and the time to breast cancer for 616 unilateral events. An excess of breast cancers occurred in the same breast during the first years of follow-up, especially in women with atypia (Fig. 4). This finding suggests that precursors to breast cancer exist in benign breast disease. Work in model systems of early steps in mammary carcinogenesis

has identified alterations in key regulatory indicators that can be studied in selected benign breast lesions.<sup>25,26</sup>

In summary, our study shows that histologic features, the age at biopsy, and the degree of family history are major determinants of the risk of breast cancer after the diagnosis of benign breast disease. We found no increased risk among women with nonproliferative lesions, unless a strong family history was present. No significant interaction between atypia and family history was apparent. The excess

risk of cancer in the ipsilateral breast in the first 10 years after the diagnosis of benign breast disease, especially in women with atypia, points to the presence of precursors in some women.

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## An Analysis of Breast Cancer Risk in Women With Single, Multiple, and Atypical Papilloma

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**Abstract:** Breast papillomas may be single or multiple and associated with atypical ductal or lobular hyperplasias (ADH/ALH). The risk of breast carcinoma development in patients with papillomas, particularly those with multiple or atypical lesions, is incompletely defined. Fibrocystic lesions were histopathologically classified in a benign breast disease cohort of 9155 who underwent biopsy from 1967 to 1991, with papilloma assessment in 9108. Individuals with papillomas (N = 480) were classified into 4 groups: single papilloma (SP, N = 372), single papilloma with ADH or ALH (SP+A, N = 54), multiple (> 5) papillomas (MP, N = 41), and multiple papillomas with ADH or ALH (MP+A, N = 13). Those without papillomas were classified as nonproliferative (NP, N = 6053), proliferative without atypia (PDWA, N = 2308), and ADH/ALH [atypical hyperplasia (AH), N = 267]. The relative risk of cancer development within our cohort was compared to that expected in the general population using standardized incidence ratios. The relative risk of breast cancer development associated with SP [2.04, 95% confidence interval (CI) 1.43-2.81] was greater than NP (1.28, 95% CI 1.16-1.42) but similar to PDWA (1.90, 95% CI 1.66-2.16). The risk associated with SP+A (5.11, 95% CI 2.64-8.92) was highly elevated but not substantively different than atypical hyperplasia (4.17, 95% CI 3.10-5.50). Patients with MP are at increased risk compared with PDWA or SP (3.01, 95% CI 1.10-6.55), particularly those with MP+A (7.01, 95% CI 1.91-17.97). There was a marginal increase in breast cancer risk (16%) among patients with proliferative disease if a papilloma was present, but this did not reach statistical significance ( $P = 0.29$ ). The observed frequency of ipsilateral (vs. contralateral) breast cancer development in papilloma subsets was not significantly different than other patient groups. We conclude that SP imparts a cancer risk similar to conventional proliferative fibrocystic change. The presence of papilloma in, or associated with, atypia does not modify the risk connotation of ADH/ALH overall. MP constitutes a proliferative breast disease subset having unique clinical and biologic behavior.

**Key Words:** intraductal papilloma, atypical hyperplasia, breast cancer

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Papillomas of the breast are defined by a constellation of pathologic findings including: (1) a discrete intraductal polypoid lesion with, (2) an arborizing fibrovascular stroma covered by a layer of myoepithelium, and (3) a second layer of columnar or cuboidal epithelium. They often form palpable nodules, reaching considerable size in some cases, although many are microscopic.<sup>4,5,12,13</sup> They are informally classified by anatomic location: central/subareolar papillomas are usually single but may reach considerable size and become symptomatic. Peripheral lesions, in contrast, are generally smaller but may be multiple and recurrent.<sup>4</sup> Papillomas are often accompanied by significant epithelial hyperplasia and/or periductal sclerosis, resulting in microscopically complex lesions.<sup>1,11,25</sup> Atypical hyperplasia (AH) may also be present within or adjacent to papilloma.<sup>19,25</sup> In these so-called atypical papillomas, the histologic distinction from ductal carcinoma in situ may be extremely problematic.

Most early investigators considered intraductal papillomas to be benign lesions without malignant potential or implied risk of developing a subsequent carcinoma.<sup>13,14</sup> More recent studies have demonstrated that these lesions, like other forms of proliferative breast disease, do increase the risk of developing carcinoma.<sup>3,6,8,9,11,25</sup> Some have suggested that papillomas may behave as direct precursor lesions.<sup>7,27</sup> Neither view, however, has been empirically tested in a sufficiently large cohort of patients with long term follow-up and appropriate population controls. Further, most studies which specifically address pathologic subsets thought to be biologically more aggressive, such as multiple papilloma or atypical papilloma, consist of relatively small numbers or are enriched by selective inclusion of cases derived from referral consult practices.<sup>19,25,28</sup>

We have recently completed pathologic evaluation of a benign breast disease cohort, consisting of all open benign breast biopsies performed at the Mayo Clinic between 1967 and 1991 (N = 9155).<sup>15</sup> All papillary lesions present in these biopsies were routinely defined as a

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component of our microscopic examination. Our objective in this study is to survey the incidence, histologic patterns and relative cancer risk associated with benign papillomas of the breast. We will specifically address the significance of multiple papillomas and papillomas with atypia and whether there is evidence to suggest they are direct precursors.

## MATERIALS AND METHODS

### Patient Selection

Patients with benign diagnoses on open excisional biopsy (OEB) of the breast, performed between January 1, 1967 and December 31, 1991, constituted the study population. Searching the Mayo Clinic Surgical Index and Pathology Index identified the cases. The details of the derivation of the study cohort have been published previously.<sup>15</sup> Briefly, subjects were excluded from the study if they met one or more of the following criteria: (1) cancer diagnosis before, at, or within 6 months of the OEB (accounting for possible occult malignancy), (2) unilateral or bilateral mastectomy or reduction before OEB, (3) refusal of research authorization, (4) no follow-up information available, or (5) slides unavailable. Of the 9155 women who met the study criteria, papilloma information for 47 were unavailable. The resulting 9108 patients constituted the study cohort, with a mean follow-up of 16 years.

### Pathology Review

A pathologist with expertise in breast pathology (D.V.) reviewed the original hematoxylin and eosin (H&E) stained slides of all cases without knowledge of original diagnosis or subsequent outcome. Cases were classified into one of three general categories: nonproliferative (NP) fibrocystic changes, proliferative changes without atypia (PDWA), and AH. NP fibrocystic changes included cyst formation, stromal fibrosis, apocrine metaplasia, and noncomplex fibroadenoma. Proliferative changes without atypia included ductal hyperplasia of usual type, sclerosing adenosis, radial scars/complex sclerosing lesions, and papilloma(s). Atypical ductal or lobular hyperplasia (ADH/ALH) constituted the AH category and the presence or absence of each was documented in each case. The criteria for classification as AH (see below) were applied to epithelial proliferations within or outside of the papilloma.

A papilloma was defined as an intraductal epithelial proliferation of any size that is supported by branching fibrovascular stalks that contains myoepithelium. In many cases they were not the predominant lesions in the biopsy specimen (eg, florid duct hyperplasia with an incidental, microscopic papilloma). Papillomas were classified as solitary (SP) or multiple (MP), the latter defined as a papillary lesion containing at least 5 papillomas in 2 nonconsecutive tissue blocks.<sup>1</sup> The presence or absence of AH (ADH and/or ALH) was also documented in the papilloma cases. If ADH or ALH was identified within the papilloma or in the surrounding

parenchyma, then the case was classified as a single papilloma with atypia (SP+A) or multiple papillomas with atypia (MP+A). For the SP+A cases, the location of the atypia (inside and/or outside the papilloma) was recorded.

ADH was defined according to the criteria of Page.<sup>23-25</sup> These lesions exhibited architecturally complex cribriformlike proliferations of monotonous cells that lacked malignant cytologic features and were confined to an area measuring < 3 mm in greatest dimension. Within papillomas, these atypical lesions only partially involved a "basement membrane bound space," with a second nonatypical population of cells composing the remainder.<sup>24</sup> ALH was defined as a proliferation of polygonal, evenly spaced cells with round, monotonous nuclei, and scant cytoplasm.<sup>23</sup> ALH was characterized by partial expansion of acini by atypical cells, often with preservation of luminal spaces, involving less than half of the acini in a lobule.

### Statistical Analysis

Data were descriptively summarized using frequencies and percentages for categorical variables, and means and standard deviations for continuous variables. We formally compared distributions of certain attributes across papilloma-defined subgroups using *t* tests and analyses of variance for the continuous variables and  $\chi^2$  tests for categorical variables.

The length of the follow-up for each woman in the study was calculated as the number of days (followed by division by 365.25 to calculate years) from her benign biopsy to the date of breast cancer diagnosis, date of death, or date of last contact. The cumulative incidence of breast cancer by papilloma status was estimated using Kaplan-Meier curves. We compared the observed number of incident breast cancer events in our cohort, stratified by papilloma status, to that expected in the general population using standardized incidence ratios (SIRs). Each individual's person years were apportioned into 5-year age and calendar period categories. Overall category-specific follow-up was then multiplied by the corresponding age-stratified and calendar period-stratified surveillance epidemiology and end results (SEER) incidence rates, and these results were then summed across all categories to calculate the expected number of events. Thus, all risk ratios account for the potentially confounding effects of age and calendar period. The Iowa SEER registry was used as the primary standard population, due to both the proximity of its participants to the Mayo Clinic catchment area and racial/ethnic similarities to our cohort.

As proliferative disease is a complex mixture of many different attributes which may synergistically affect the risk of breast cancer, it is possible that other forms of proliferative change could confound the association of papillomas and breast cancer. Thus, we sought to assess the independent modifying effects of different forms of proliferation using Poisson regression analyses, modeling the individual-specific, log-transformed expected event

rate as the offset term. This approach facilitates the calculation of SIRs with the added flexibility that generalized linear models provide, such as covariate adjustment and formal assessment of heterogeneity. The Iowa SEER registry was again used to calculate the expected event rates. Analyses were subset to women with proliferative disease. Based on the Poisson models, we calculated relative SIRs (rSIRs), directly comparing ratios of SIRs across levels of each of the proliferation attributes. The accompanying *P* values assess the heterogeneity of breast cancer SIRs across levels of the attribute. The following types of proliferation were examined: presence of atypia, presence of papillomas, presence of sclerosing adenosis, and presence of radial scars. Two sets of poisson models were fit: one that accounted only for the effects of age and calendar period, and one that accounted additionally and simultaneously for the effects of the other proliferation attributes.

We compared the potentially differential risk of ipsilateral versus contralateral breast cancer within the cohort across papilloma-defined subgroups using a competing risk approach, based on the Poisson distribution. Women with missing biopsy or cancer side information, or with benign breast disease (BBD) or cancer diagnosed bilaterally, were excluded from these analyses. For each subgroup, we compared the incidence rate for ipsilateral cancer to the corresponding rate for contralateral cancer. When calculating incidence for ipsilateral cancer, individuals with contralateral cancer were censored at their date of diagnosis, and vice versa. This approach yields identical person years for each event type, reducing comparisons of incidence to simple comparisons of counts via  $\chi^2$  tests of significance. All statistical tests were 2-sided, and all analyses were carried out using the SAS software system (SAS Institute, Inc, Cary, NC).

## RESULTS

### Pathologic Findings

The Mayo benign breast disease cohort has been presented in detail elsewhere.<sup>15</sup> Our current cohort includes 9108 patient biopsies, classified as follows: 6053 (66.5%) NP; 2308 (25.3%) PDWA; and 267 (2.9%) AH. Papilloma was identified in 480 (5.3%) of the biopsies,

distributed within the PDWA and AH categories. The majority of papillomas (372, 4.1%) were SP. There were 54 SP+A (0.6%), 41 MP (0.5%), and 13 (0.1%) MP+A. The mean age at biopsy was youngest for the NP group (49.9, SD 14.8), intermediate for the nonatypical proliferative groups [PDWA 53.6 (SD 12.1), SP 55.2 (SD 14.5) and MP 53.9 (SD 15.5)], and oldest for the atypical groups [AH 57.3 (SD 11.6), SP+A 59.1 (SD 13.4), and MP+A 65.1 (SD 14.0)].

Because family history is a known risk factor for the development of carcinoma, we compared the frequency of papilloma status with family history to determine if there were any differences among the subsets. Family history was available in 4846 (53%) of the 9108 cases. As Table 1 illustrates, the majority of cases in all subsets did not have a family history of breast cancer. The remainder of the cases varied from a weak to strong family history.  $\chi^2$  tests revealed no differences in distribution of papilloma(s) across levels of family history (*P* = 0.49).

Papillomas were accompanied by a complex mixture of proliferative changes. Sclerosing adenosis and usual ductal hyperplasia were both present in at least 50% of cases from each papilloma subgroup (Table 2). Radial scars also occurred at significantly increased frequency (16% SP, 33% SP+A, 34% MP, 31% MP+A) compared to individuals without papillomas (4%, *P* < 0.001). Among papilloma cases, radial scars were significantly more common in SP+A, MP, and MP+A compared with SP (*P* < 0.001,  $\chi^2$  test).

With respect to atypia in the setting of papilloma, most cases (33/51, 65%) contained ADH alone. There were 6 (12%) with ALH and 12 with both ADH and ALH (23%). Examples of atypical papilloma are illustrated in Figures 1 to 4. Of the 45 cases with ADH, atypia was present within the papilloma in 16 (36%), outside of the papilloma in 17 (38%), and present both inside and outside the papilloma in 12 (26%). One SP+A case consisted of 2 biopsies, one contained a solitary papilloma (left breast) and the other ADH (right breast). For purposes of this study, this case was classified as atypia outside of the papilloma.

### Outcome

Among the overall Mayo cohort, the relative risk of developing carcinoma was: NP 1.3 [95% confidence

TABLE 1. Comparison of Family History of Breast Cancer Across the Various Papilloma Subtypes

Diagnosis	Family History of Breast Cancer <sup>a</sup>			
	None N (%)	Weak N (%)	Strong N (%)	Total N (%)
No papilloma	2549 (52.60)	1119 (23.09)	911 (18.80)	4579 (94.49)
SP	105 (2.17)	50 (1.03)	41 (0.85)	196 (4.04)
SP+A	16 (0.33)	7 (0.14)	11 (0.23)	34 (0.70)
MP	18 (0.37)	6 (0.12)	6 (0.12)	30 (0.62)
MP+A	4 (0.08)	0 (0.00)	3 (0.06)	7 (0.14)

<sup>a</sup>Family history was missing in 4262 cases.

TABLE 2. Frequency of Proliferative Breast Disease Across the Papilloma Subtypes

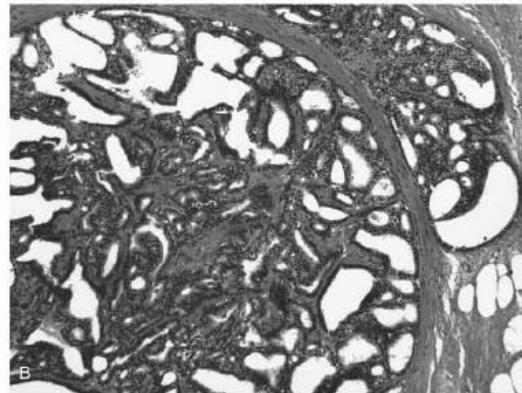
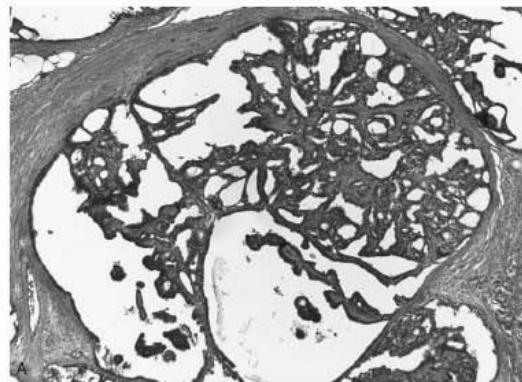
	Ductal Hyperplasia		Sclerosing Adenosis		Radial Scars	
	Yes N (%)	No N (%)	Yes N (%)	No N (%)	Yes N (%)	No N (%)
No papilloma*	1569 (18)	7055 (82)	2000 (23)	6625 (77)	339 (4)	8280 (96)
SP†	241 (64)	131 (36)	207 (56)	164 (44)	59 (16)	312 (84)
SP+A	52 (96)	2 (4)	38 (70)	16 (30)	18 (33)	36 (67)
MP	38 (93)	3 (7)	39 (95)	2 (5)	14 (34)	27 (66)
MP+A	13 (100)	0 (0)	8 (62)	5 (38)	4 (31)	9 (69)

Ductal hyperplasia includes moderate and florid ductal epithelial hyperplasia.

\*This includes NP, proliferative without atypia, and AH cases. Four observations missing values for ductal hyperplasia, 9 for radial scars, and 3 for sclerosing adenosis.

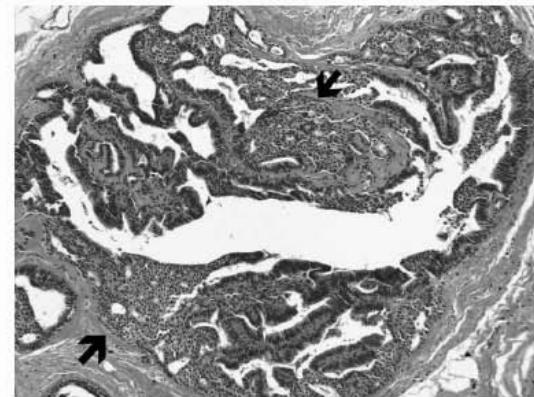
†One observation missing value for sclerosing adenosis and radial scar.

interval (CI), 1.2-1.4], PDWA 1.9 (95% CI 1.7-2.2), and AH 4.4 (95% CI 3.4-5.6). Overall, 724 (8%) of the patients in the cohort have developed breast carcinoma.

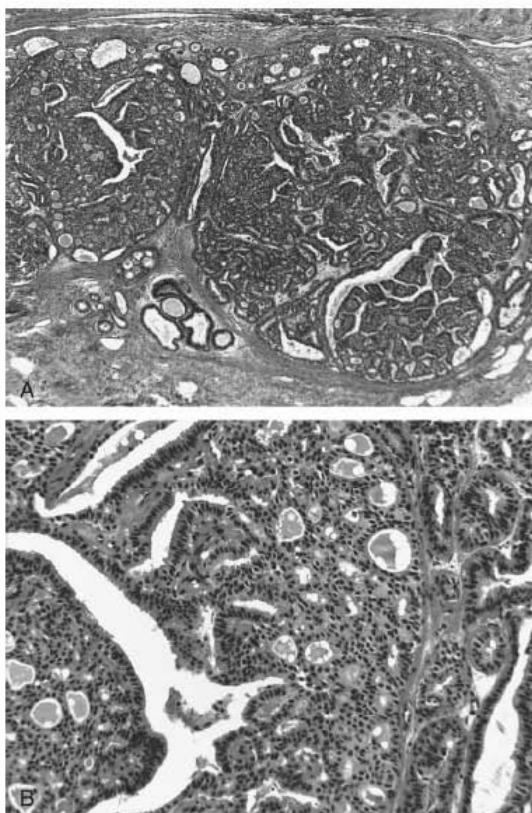


**FIGURE 1.** Low magnification scanning micrograph showing an architecturally complex papillary lesion containing fibrovascular stroma with focal cribriform growth. At higher magnification areas of cribriformlike architecture can be appreciated at the periphery of the lesion. Lack of uniform involvement and bland cytologic features preclude a diagnosis of ductal carcinoma in situ.

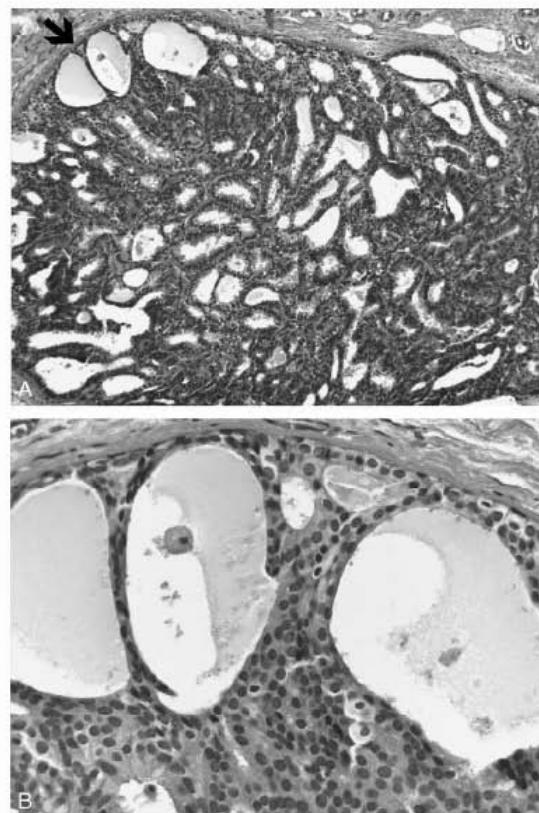
Table 3 summarizes the mean age at biopsy, mean interval to development of breast cancer, and risk of carcinoma development among the histopathologic groups with respect to papilloma status. Patients lacking a papilloma(s) who had proliferative disease without atypia had a relative risk of 1.90 (95% CI 1.66-2.16) of developing cancer compared to the control population. Patients with a solitary papilloma without atypia had a risk of 2.04 (95% CI 1.43-2.81), roughly equivalent to other forms of proliferative disease without atypia. Patients with AH and no papilloma(s) had a relative risk of 4.17 (95% CI 3.10-5.50). Individuals with a SP+A had a risk of 5.11 (95% CI 2.64-8.92), slightly greater than those with AH lacking a papillary lesion. The breast cancer risk for multiple papillomas without atypia fell between proliferative disease without atypia and AH (3.01, 95% CI 1.10-6.55). Multiple papilloma cases with atypia had the greatest likelihood of developing cancer, with a relative risk of 7.01 (95% CI 1.91-17.97). A Kaplan-Meier curve depicting cumulative incidence of breast cancer among all histopathologic groups is



**FIGURE 2.** Atypical papilloma (low magnification). Most areas are comprised of columnar epithelium on fibrovascular stalks. At least 2 foci (arrows) show a monotonous cellular proliferation lacking stroma and containing small secondary lumens.



**FIGURE 3.** At scanning magnification this papilloma is characterized by marked hypercellularity and variable architecture. Higher magnification photomicrograph highlighting confluent growth of epithelium with partially developed cribriform architecture.



**FIGURE 4.** The architecture is primarily microglandular, but focal complex growth may also be appreciated (arrow). Higher magnification of cribriform area. Lack of uniform involvement and low grade cytology preclude a diagnosis of *in situ* carcinoma.

presented in Figure 5. The mean interval to cancer development was greater than 5 years in all histologic groups except MP 4.8 (SD 3.2). It was longest in patients with NP 8.7 (SD 7.2). There was no difference in mean interval to cancer between AH and SP+A [6.5 (SD 5.3) vs. 6.2 (SD 4.7),  $P = 0.87$ ,  $t$  test].

As papillomas were most frequently identified in the setting of other proliferative lesions, we attempted to determine the degree to which the apparent breast cancer risk seen in these patients was directly attributable to the papilloma(s), versus other coexisting forms of proliferation present within the breast. We performed a univariate analysis using Poisson regression models subset to only those women with proliferative changes (ie, NP cases were excluded). After accounting for age and calendar period, women with some form of proliferative disease (eg, AH, radial scar, or sclerosing adenosis) and with a papilloma, had a relative risk of breast cancer roughly 20% higher

than those without a papilloma. Thus, within the group of patients with proliferative disease, the presence of a papilloma marginally increased risk. However, this result did not reach statistical significance ( $P = 0.17$ ). As the univariate model accounted for the excessive risk due to proliferative disease, but it did not adjust for the effects of other individual forms of proliferation, we performed a multivariate analysis adjusting for AH, ductal hyperplasia, sclerosing adenosis, and radial scars. Results were similar to the univariate model: the presence of papillomas increased risk by an additional 16% over those patients without a papilloma. However, this result again failed to reach statistical significance ( $P = 0.29$ ). We also performed the multivariate analysis subset to proliferative cases without atypia. After adjusting for sclerosing adenosis, radial scars, and duct hyperplasia, the presence of papilloma increased risk by an additional 10% over those patients without a papilloma ( $P = 0.42$ ).

**TABLE 3.** Demographic Characteristics and SIRs of Breast Carcinoma Development for the Mayo Cohort Compared With the Iowa SEER Registry

Diagnosis	Overall N	Age at Biopsy (y) Mean (SD)	Time to Cancer (y) Mean (SD)	Observed Cancers	Expected Cancers	SIR (95% CI)
No papilloma present						
NP	6053	49.9 (14.8)	8.7 (7.2)	383	298	1.28 (1.16-1.42)
Proliferative	2308	53.6 (12.1)	7.8 (6.4)	232	122	1.90 (1.66-2.16)
AH	267	57.3 (11.6)	6.5 (5.3)	50	12	4.17 (3.10-5.50)
Papilloma present						
SP	372	55.2 (14.5)	5.9 (5.0)	37	18	2.04 (1.43-2.81)
SP + A	54	59.1 (13.4)	6.2 (4.9)	12	2	5.11 (2.64-8.92)
MP	41	53.9 (15.5)	4.8 (3.2)	6	2	3.01 (1.10-6.55)
MP + A	13	65.1 (14.0)	5.8 (3.8)	4	1	7.01 (1.91-17.97)

The "Overall" column refers to the total number of cases in each group. Analyses account for the effects of age and calendar period.

In the SP + A patients, risk for breast cancer was not associated with the microscopic location of ADH. Cancers developed in 25% (4/16) of patients with ADH within papilloma compared with 29% (5/17) patients with ADH outside of the papilloma and 17% (2/12) with ADH in both locations. None of the patients with ALH alone (N = 6) has yet developed breast carcinoma.

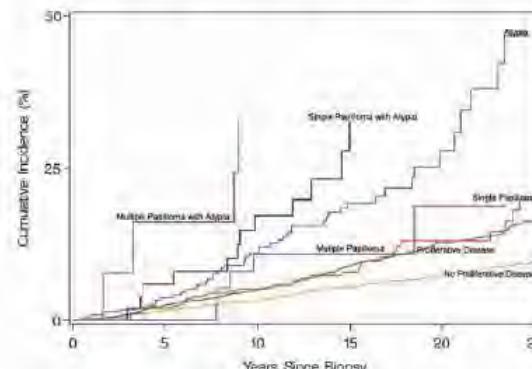
Table 4 summarizes side of cancer development in relation to the side of the original excisional biopsy. With respect to the overall Mayo cohort, 56% of the patients in the NP, PDWA, and AH groups developed carcinoma in the ipsilateral (same) breast as the biopsy.<sup>15</sup> Sixty-five percent of the carcinomas in the papilloma group developed in the ipsilateral breast. The likelihood for development of ipsilateral cancer among those with papilloma compared with nonpapilloma groups, was not statistically different ( $P = 0.33$ ,  $\chi^2$  test).

## DISCUSSION

Our study defines the incidence, spectrum of pathology, and breast cancer risk attributable to benign

papillomas that were identified in a large cohort of consecutive, nonselected benign biopsies. It is the first to specifically address the cancer risk associated with papillomas, either with or without atypia, using epidemiologically valid comparisons between carefully defined pathologic subsets including nonpapilloma proliferative lesions. The data demonstrate that presence of a single papilloma without atypia conveys an overall breast cancer risk that is similar to or marginally greater than other commonly recognized proliferative fibrocytic lesions. It is unclear, even after multivariate statistical analysis, whether this small difference is due to the more frequent presence of other proliferative lesions in those with papillomas. Second, the presence of ADH/ALH in association with a single papilloma ("atypical papilloma"), does not appreciably modify the risk connotation attributable to atypia overall. Finally, the follow-up data from the cohort imply that patients with multiple papillomas are at a significantly elevated risk for breast cancer, even if atypia is not identified in their biopsy.

The Mayo benign breast disease cohort is derived from the surgical practice at one institution and is not



**FIGURE 5.** Kaplan-Meier curve illustrating cumulative incidence of cancer development among the histologic groups in the Mayo cohort.

**TABLE 4.** Breast Cancer Sidedness Among the Different Diagnostic Groups With Respect to Excisional Biopsy Location

Diagnosis	Number of Cancers*	Contralateral N (%)	Ipsilateral N (%)
All Subjects			
NP	354	163 (46)	191 (54)
Proliferative	234	107 (46)	127 (54)
AH	57	22 (30)	35 (61)
No papilloma present			
NP	354	163 (46)	191 (54)
Proliferative	194	93 (48)	101 (52)
AH	43	17 (40)	26 (60)
Papilloma present			
SP	34	10 (29)	24 (71)
SP + A	11	4 (36)	7 (64)
MP/MP + A	9	5 (56)	4 (44)

MP and MP + A cases were summed because of the small number of events.

\*Individuals with missing side information, bilateral BBD, or bilateral cancer have been removed from the analysis.

enriched with extramural pathology consultation materials. Indications for breast biopsy were not different than those employed by comparable institutions during the survey period. To our knowledge, it is the largest benign breast disease cohort that has been subject to standard pathologic review.<sup>2,8-10,17,18,21,26</sup> The size of the cohort and long follow-up allow for robust statistical analysis. The observed proportion of cases and relative risks of carcinoma development associated with NP, proliferative, and atypical lesions is similar to other large surveys of benign breast disease.<sup>5,9,17,16,20,21</sup>

Benign papillomas constitute an important subset of mammary fibrocystic change, collectively accounting for about 5% of proliferative cases overall. As seen in this study, moreover, papillomas are frequently accompanied by a complex of other proliferative lesions, particularly adenosis and duct hyperplasia. Radial scars are also often present, especially in the SP+A, MP and MP+A subsets (31% to 34%). Finally, papillomas often comprise a background on which atypias develop; 20% of all atypias in our BBD cohort overall were present in cases that also had papillomas. Conversely, about 14% of biopsies with papilloma(s) contained ADH and/or ALH. The observed associations with adenosis and radial scarring are noteworthy in the sense that both are characterized by combined proliferation of epithelial and nonepithelial populations (myoepithelial cells and fibroblasts, respectively). These findings imply that breast tissues harboring papillomas may be characterized by simultaneous activation/proliferation of divergent cell populations. It may be hypothesized that the background of multilineage cell proliferation reflects an especially permissive environment for development of hyperplastic lesions, accounting for the markedly cellular character of this BBD subset, and possibly for the more frequent evolution of atypias.

Although papillomas are often associated with other proliferative or atypical lesions, it is nonetheless true that most benign biopsies with papillomas—about 78%—contained single papillomas and lacked ADH/ALH. Within this subset (ie, single papilloma without atypia) the age at diagnosis and overall breast cancer risk were quite similar to patients with proliferative lesions overall—53.6 versus 55.2 years and 1.90 (95% CI 1.66-2.16) versus 2.04 (95% CI 1.43-2.81), respectively. On the basis of these data we would not advise the risk classification of single, nonatypical papillomas apart from other proliferative fibrocystic lesions.

Some may hypothesize that atypical proliferations developing within papillomas represent biologically distinct, direct precursor lesions. However, the simultaneous presence of papilloma with ADH or ALH (SP+A) was associated with a breast cancer risk (5.11, 95% CI 2.64-8.92) that was similar to, or marginally elevated, relative to other atypias in our cohort (4.17, 95% CI 3.10-5.50). Importantly, in the papilloma cases we failed to identify significant tendency to ipsilateral breast cancer development or short interval to breast cancer diagnosis. Thus, we identify no convincing evidence to suggest that these lesions constitute an homogeneous group of direct cancer

precursors. We also attempted to discern whether the location of atypia relative to a papilloma had special significance. Although the number of cases limits definitive interpretation, our data imply that geographic location of ADH relative to a papillary lesion would not necessarily be a clinically useful indicator of breast cancer risk apart from other parameters. However, our data would not support the practice of separately denoting an atypical papilloma as a distinct subset of ADH.

Page et al<sup>25</sup> have published a nested case control survey that compares breast cancer risk in 122 patients with papillomas. Their incidence of atypia occurring within or in association with papilloma (17/122, 14%) was similar to our series (54/480, 11%) as was the time interval between biopsy and subsequent breast malignancy. However, the absolute risk of breast cancer after atypical papillomas in the Page et al study was 53% (9/17). In contrast, in our study only 22% of such individuals (12/54) had developed breast cancer at 16 years. They also observed a significantly elevated relative breast cancer risk (2.30 to 3.35×) attributable to papilloma that was modified by presence of concurrent atypia (4.40 to 13.10×). Because we employed similar diagnostic criteria, we ascribe the differences with our study to their relatively limited number of cases and the study design (ie, case control vs. cohort).

Haagensen<sup>12</sup> and Murad<sup>22</sup> have emphasized the unique clinical behavior of MP, noting from selected series of cases that these patients have significantly elevated breast cancer risk. The incidence and relative cancer risk of MP, however, has not been previously described. Our data show that MP cases constitute a rare subset, accounting for 0.6% of BBD patients. However, depending on the presence of atypical lesions, MP patients have a breast cancer risk that is 3 to 7 times greater than age matched women in the population overall. Thus, our data indicate that MP, even without concurrent atypia, convey a relative risk between proliferative disease overall and AH. On the basis of these findings, we recommend that MP should receive wider recognition as a diagnostic entity and that these patients should be, at a minimum, followed carefully.

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# ARTICLES

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## Age-Related Lobular Involution and Risk of Breast Cancer

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**Background:** As women age, the lobules in their breasts undergo involution or regression. We investigated whether lobular involution in women with benign breast disease was associated with subsequent breast cancer risk. **Methods:** We examined biopsy specimens of 8736 women in the Mayo Benign Breast Disease Cohort from whom biopsy samples were taken between January 1, 1967, and December 31, 1991. Median follow-up for breast cancer outcomes was 17 years. We classified lobular involution in the background breast tissue as none (0% involuted lobules), partial (1%–74%), or complete ( $\geq 75\%$ ). Subsequent breast cancer events and data on other risk factors were obtained from medical records and follow-up questionnaires. To estimate relative risks (RRs), standardized incidence ratios were calculated by use of incidence rates from the Iowa Surveillance, Epidemiology, and End Results (SEER) Registry. All statistical tests were two-sided. **Results:** Distribution of extent of involution was none among 1627 (18.6%) women, partial among 5197 (59.5%), and complete among 1912 (21.9%). Increased involution was positively associated with increased age and inversely associated with parity (both  $P < .001$ ). The relative risk for the entire cohort of 8736 women, compared with the Iowa SEER population, was 1.40 (95% CI = 1.30 to 1.51). Risk of breast cancer was associated with the extent of involution (for no involution, RR [i.e., observed versus expected] = 1.88, 95% confidence interval [CI] = 1.59 to 2.21; for partial involution, RR = 1.47, 95% CI = 1.33 to 1.61; and for complete involution, RR = 0.91, 95% CI = 0.75 to 1.10; test for heterogeneity  $P < .001$ ). Lobular involution modified risk in all subsets (e.g., among women with atypia, for no involution, RR = 7.79, 95% CI = 3.56 to 14.81; for partial involution, RR = 4.06, 95% CI = 3.03 to 5.33; and for complete involution, RR = 1.49, 95% CI = 0.41 to 3.82;  $P = .003$ ). **Conclusions:** In this large cohort of women with benign breast disease, lobular involution was associated with reduced risk of breast cancer. Aberrant involution may be a biologically important phenomenon in breast cancer biology. [J Natl Cancer Inst 2006;98:1600–7]

The mammary gland undergoes profound physiologic changes throughout the phases of a woman's life, including puberty, pregnancy, lactation, postlactational involution, and aging (1,2). The epithelial tissue of the human breast is organized into 15–20 major lobes, each made up of lobules that contain the milk-forming acini. As a woman ages, breast lobules regress, or involute, with a reduction in the number and size of

acini per lobule and replacement of the delicate intralobular stroma with the more dense collagen of breast connective tissue (Fig. 1) (1,3–5). Over time, there is progressive fatty replacement of glandular elements and collagen (1,5). This process differs greatly from postlactational involution. After lactation, there is regression of all breast tissues as secretory activity is curtailed, but there is no substantive loss of glandular tissue (1,2).

Although involution of the breast involves a consistent sequence of histologic changes, the rate and extent of involution vary considerably among individual women (3,4). This age-related lobular involution has been documented in women younger than age 40 years and thus involves factors not limited to the onset of menopause (1,3–5). Cowan et al. (4) studied age-related involution in breast tissue obtained at autopsy and speculated that obstetrical and lactational history may be more important than age at influencing the onset of involution, but they did not provide specific obstetrical data to support their claim. Geschickter (5) studied more than 100 breast specimens obtained at autopsy or surgery and noted the degree of involution by age and obstetrical history. He observed early changes consistent with lobular involution in 33% of women younger than age 40 years. He also found that repeated pregnancies were associated with the persistence of lobules, whereas lobule size and number declined in the absence of childbearing.

It has been hypothesized that age-related lobular involution, with its loss of glandular elements, may be associated with a decreased risk of breast cancer (6,7). To test this hypothesis, we characterized the extent of lobular involution by age group in a large cohort of women with benign breast disease and examined the association between involution and breast cancer risk.

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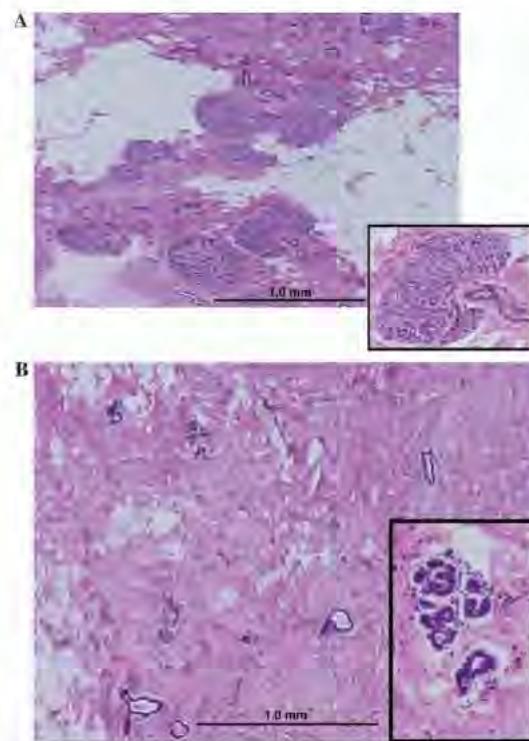
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See "Notes" following "References."

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**Fig. 1.** Histologic features of age-related involution. A) Preinvolutional breast tissue with multiple intact terminal duct lobular units, each composed of multiple acini and specialized stroma (Inset). B) Complete lobular involution with mostly residual ducts with residual terminal duct lobular units, largely depleted of acini (Inset). Scale bars = 1.0 mm.

## PARTICIPANTS AND METHODS

### Study Population

Inclusion and exclusion criteria for the study cohort have been previously described (8). Briefly, the study population consisted of women aged 18–85 years who had benign breast disease (i.e., a breast biopsy examination with benign findings) diagnosed via surgical excision at Mayo Clinic between January 1, 1967, and December 31, 1991. The Mayo Benign Breast Disease Cohort included 9087 women with 15 years of follow-up at initial report (8). Since that report, we obtained cancer follow-up data for an additional 289 women, for whom that information was lacking previously, bringing the cohort to a total of 9376 women with a median of 17 years of follow-up. For 640 breast biopsy samples, the biopsy specimen consisted entirely of the index lesion; there was no background breast tissue in which to determine the degree of lobular involution. Thus, the final cohort for this analysis included 8736 women.

All protocol procedures and patient contact materials were reviewed and approved by the Institutional Review Board of the Mayo Clinic. Return of the patient contact materials was considered implied consent.

### Histology

All slides were reviewed by a breast pathologist (DWV) without knowledge of patient age, cancer outcome, or original histologic diagnosis. Biopsy findings were classified by the most extreme degree of hyperplasia as nonproliferative, proliferative disease without atypia, or atypical hyperplasia, as previously reported (8).

Each biopsy specimen was also categorized according to the extent of lobular involution in the background breast tissue. Involved terminal duct lobular units (TDLUs) contain only a few to several small acini that may be distended by cystic change (Fig. 1). Involved lobules also have flattened inconspicuous acinar epithelium with fibrosis of specialized intralobular stroma. The degree of involution for each specimen was categorized as none (0% TDLUs involved), partial (1%–74% TDLUs involved), or complete ( $\geq 75\%$  TDLUs involved). These cut points were set by the pathologists at the initiation of the study to best distinguish the extremes of no involution from near-complete involution.

In general, viewing five to six lobules was sufficient to assess the extent of involution. One slide from a breast specimen typically contained a dozen or more lobules. There are two exceptions to this statement: 1) when involution was extensive and there are only a few lobular remnants on the slide (which is sufficient to state that complete lobular involution has occurred) and 2) when the entire sample consists of an epithelial hyperplastic lesion, as was the case for 640 (6.8%) of the 9376 women in our original cohort.

### Risk Factor Information and Follow-up

To obtain information about family history, reproductive history, and use of hormone replacement therapy, a study-specific questionnaire was sent to patients; 5352 (61%) of the 8736 women or their next of kin returned the questionnaire. Follow-up for breast cancer events was obtained through comprehensive (inpatient and outpatient) Mayo medical records and the questionnaire.

Family history of breast cancer was categorized as strong, weak, or negative. A strong family history was defined as the patient having 1) at least one first-degree relative with breast cancer diagnosed before age 50 years or 2) two relatives with breast cancer at any age, with at least one being a first-degree relative. Patients with family history of breast cancer who did not meet the above criteria were categorized as having a weak family history (8).

### Statistical Analysis

Data were summarized descriptively by use of frequencies and percentages. We initially compared the unadjusted distribution of breast cancer risk factors across levels of involution with chi-square tests of statistical significance. Subsequent comparisons were made after accounting for the effects of age by use of multivariate, nominal logistic regression analysis (9). We summarized results from these analyses by use of adjusted percents, carried out by calculating log odds estimates for each 10-year age category (<40, 40–49, 50–59, 60–69, 70–79, or  $\geq 80$  years), back-transforming to percent estimates, and then averaging the corresponding percents across all sets of age. This approach was similar to a least-squares means estimate in an analysis of variance setting. Among 245 women with synchronous bilateral biopsy examinations, we assessed the level of agreement across the two readings by use of weighted kappa statistics and their corresponding 95% confidence intervals (CIs).

The length of follow-up for each woman in the study was calculated as the number of days from her biopsy examination to the date of her breast cancer diagnosis, death, or last contact. We estimated relative risks (RRs) on the basis of standardized incidence ratios by dividing the observed numbers of incident breast cancers by expected numbers of population-based incident breast cancers. Expected values were calculated by apportioning each woman's person-years of follow-up into 5-year age and calendar-period categories and multiplying these by the corresponding breast cancer incidence rates from the Iowa Surveillance, Epidemiology, and End Results (SEER) Registry. This reference population was chosen because of its demographic similarities to the Mayo Clinic population (80% of cohort members reside in the upper Midwest). Potential heterogeneity in relative risks across levels of involution was assessed by use of Poisson regression analysis, with the log-transformed expected event rate for each individual modeled as the offset term.

In addition to assessing overall breast cancer risk, we also compared rates of ipsilateral to contralateral breast cancer in relation to the side of the benign lesion, both overall and by levels of involution. When calculating incidence for ipsilateral cancer, individuals with contralateral cancer were censored at their date of diagnosis, and vice versa. Women with missing laterality information, bilateral biopsy examination results, or bilateral breast cancer were censored for both events in these analyses. This approach yielded identical numbers of person-years for each type of event. As a result, the length of follow-up was not a factor in the analysis, and the rate comparisons reduced to simple comparisons of the number of events. Thus, we were able to assess whether the relative rate of ipsilateral cancer (compared with contralateral cancer) differed across levels of involution using simple chi-square tests of statistical significance. All statistical tests were two-sided, and all analyses were carried out with the SAS software system (SAS Institute, Inc, Cary, NC).

## RESULTS

### Extent of Lobular Involution

We characterized the extent of lobular involution in the benign breast biopsies of a cohort of 8736 women with tissue sampled between January 1, 1967, and December 31, 1991, at the Mayo Clinic. The distribution of the patients by level of lobular involution was as follows: no involution among 1627 (18.6%) women, partial involution among 5197 (59.5%) women, and complete involution among 1912 (21.9%) women.

### Factors Associated With Involution

As shown in Table 1, the degree of lobular involution increased progressively with age at diagnosis of benign breast disease ( $P<.001$ ). Complete involution of lobular units was observed in only 19 (3.4%) of the 566 women who were younger than 30 years at their benign biopsy; in 53 (5.1%) of the 1037 women aged 30–39 years; in 142 (5.8%) of the 2446 women aged 40–49 years; in 455 (21.6%) of the 2109 women aged 50–59 years; in 724 (45.3%) of the 1600 women aged 60–69 years; and in 519 (53.1%) of the 978 women aged 70 years or older. The gradual nature of the involution process is apparent in that it is already present at least to a partial degree in more than half of the women younger than 40 years and is still ongoing in women older than 70 years.

**Table 1.** Association of lobular involution with age at diagnosis of benign breast disease, family history of breast cancer, parity, lactation, and hormone replacement therapy<sup>a</sup>

Characteristic	Extent of lobular involution, No. (%)			<i>P</i> value <sup>†</sup>
	None	Partial	Complete	
Overall	1627 (18.6)	5197 (59.5)	1912 (21.9)	
Age at BBD, y				<.001
18–29	308 (54.4)	239 (42.2)	19 (3.4)	
30–39	417 (40.7)	567 (54.7)	53 (5.1)	
40–49	643 (26.3)	1661 (67.9)	142 (5.8)	
50–59	218 (10.3)	1436 (68.1)	455 (21.6)	
60–69	29 (1.8)	847 (52.9)	724 (45.3)	
≥70	12 (1.2)	447 (45.7)	519 (53.1)	
Family history of breast cancer <sup>‡</sup>				<.001
None or weak	796 (21.1)	2717 (56.4)	911 (22.4)	
Strong	223 (24.5)	566 (55.9)	139 (19.5)	
Parity <sup>§</sup>				<.001
Nulliparous	113 (17.6)	421 (55.3)	177 (27.1)	
Parous	893 (22.6)	2780 (56.2)	836 (21.2)	
No. of children <sup>§</sup>				<.001
0	113 (17.6)	421 (55.3)	177 (27.1)	
1	75 (17.2)	269 (54.7)	131 (28.0)	
2	324 (22.2)	891 (56.2)	258 (21.5)	
3	257 (23.9)	742 (55.8)	205 (20.4)	
≥4	235 (26.6)	871 (55.3)	242 (18.0)	
Children breastfed <sup>  </sup>				.428
No	464 (21.6)	1555 (57.4)	436 (21.1)	
Yes	431 (23.3)	1202 (55.9)	364 (20.8)	
Hormone replacement therapy <sup>¶</sup>				.016
Ever	481 (22.5)	1330 (57.1)	494 (20.3)	
Never	516 (21.8)	1837 (55.4)	458 (22.9)	

<sup>a</sup>For age at diagnosis of benign breast disease (BBD), percentage values were unadjusted. For all other variables, percentage values were adjusted for age.

<sup>†</sup>For age at BBD, *P* values were calculated using chi-square tests of statistical significance. For all other variables, *P* values were calculated by use of multilevel logistic regression analyses, accounting for the effects of age. All statistical tests were two-sided.

<sup>‡</sup>Information on family history was available for 5352 of 8736 women.

<sup>§</sup>Information on parity was available for 5220 of 8736 women. Specific number of children was available for 4500 of the 4509 parous women.

<sup>||</sup>Information on breastfeeding was available for 4452 of 8736 women.

<sup>¶</sup>Information on hormone replacement therapy was available for 5116 of 8736 women.

We also found a strong, inverse association ( $P<.001$ ) between lobular involution and parity (Table 1). Specifically, the likelihood of complete involution was 27.1% (95% CI = 24.1% to 30.1%) in nulliparous women, 28.0% (95% CI = 24.7% to 31.4%) in women who had one child, 21.5% (95% CI = 19.3% to 23.8%) in women who had two children, 20.4% (95% CI = 17.8% to 23.0%) in women who had three children, and 18.0% (95% CI = 16.1% to 20.0%) in women who had four children or more.

Separating women into categories of ever versus never breastfeeding did not reveal any relationship with extent of lobular involution ( $P = .428$ ). Women who reported having used hormone replacement therapy were slightly less likely to have complete involution (20.3%) than those with no history of hormone replacement therapy use (22.9%) ( $P = .016$ ). Breast tissue from women with a strong family history of breast cancer was less likely than that from women with no or a weak family history of breast cancer to demonstrate lobular involution; i.e., after adjustment for age, more women with a strong family history had no involution (24.5%) than those with no or a weak family history

**Table 2.** Association of involution and other risk factors with breast cancer after the diagnosis of benign breast disease

Characteristic	No. of women	No. of person-years	No. of observed events	No. of expected events*	Relative risk (95% CI)†
Degree of involution					
None (0%)	1627	32 271	150	79.6	1.88 (1.59 to 2.21)
Partial (1%–74%)	5197	90 409	440	300.1	1.47 (1.33 to 1.61)
Complete (≥75%)	1912	28 376	106	116.5	0.91 (0.75 to 1.10)
Histologic type					
Nonproliferative	5736	101 201	355	321.5	1.10 (0.99 to 1.23)
Proliferative without atypia	2677	45 418	276	158.1	1.75 (1.55 to 1.96)
Proliferative with atypia	323	4436	65	16.6	3.91 (3.02 to 4.98)
Age at biopsy, y					
<45	2682	52 055	158	108.4	1.46 (1.24 to 1.70)
45–55	2559	49 246	254	169.0	1.50 (1.32 to 1.70)
≥55	3495	49 754	284	218.8	1.30 (1.15 to 1.46)
Family history of breast cancer‡					
None or weak	4424	81 514	329	269.4	1.22 (1.09 to 1.36)
Strong	928	18 385	115	59.5	1.93 (1.59 to 2.32)
Age at birth of first live child, y§					
Nulliparous	711	13 021	71	41.1	1.73 (1.35 to 2.18)
<30	4121	77 710	327	257.9	1.27 (1.13 to 1.41)
≥30	388	7091	30	22.4	1.34 (0.90 to 1.92)

\*Number of events expected on the basis of Iowa Surveillance, Epidemiology, and End Results breast cancer incidence data.

†All analyses account for the effects of age and calendar period. CI = confidence interval.

‡Information on family history of breast cancer was available for 5352 of 8736 women.

§Information on parity was available for 5220 of 8736 women.

(21.1%), and fewer women with a strong family history had complete involution (19.5%) than those with no or a weak family history (22.4%) (logistic regression analysis comparing distribution of involution across levels of family history,  $P<.001$ ).

We also examined the extent of lobular involution by category of benign breast disease. Among women with nonproliferative disease, 27.2% had complete involution. However, among women with proliferative disease without or with atypia, only 11.5% had complete lobular involution ( $P<.001$ ).

#### Lobular Involution and Breast Cancer Risk

This cohort of women with benign breast disease was, overall, at increased risk of breast cancer when compared with age-matched women in the general population. Specifically, the relative risk for the entire cohort of 8736 women, compared with the Iowa SEER population, was 1.40 (95% CI = 1.30 to 1.51). In our cohort, degree of involution was associated inversely with breast cancer risk (Table 2; e.g., for no involution, RR = 1.88, 95% CI = 1.59 to 2.21; for partial involution, RR = 1.47, 95% CI = 1.33 to 1.61; and for complete involution, RR = 0.91, 95% CI = 0.75 to 1.10; test for heterogeneity  $P<.001$ ).

Figure 2 illustrates the observed associations between the extent of involution and breast cancer risk among strata of age, histology, family history, and parity. Extent of lobular involution modified age-related breast cancer risk (e.g., for a woman older than 55 years with no involution, RR = 3.21, 95% CI = 1.90 to 5.08, and for a similar woman with complete lobular involution, RR = 0.92, 95% CI = 0.74 to 1.14). The same pattern was observed in all age groups.

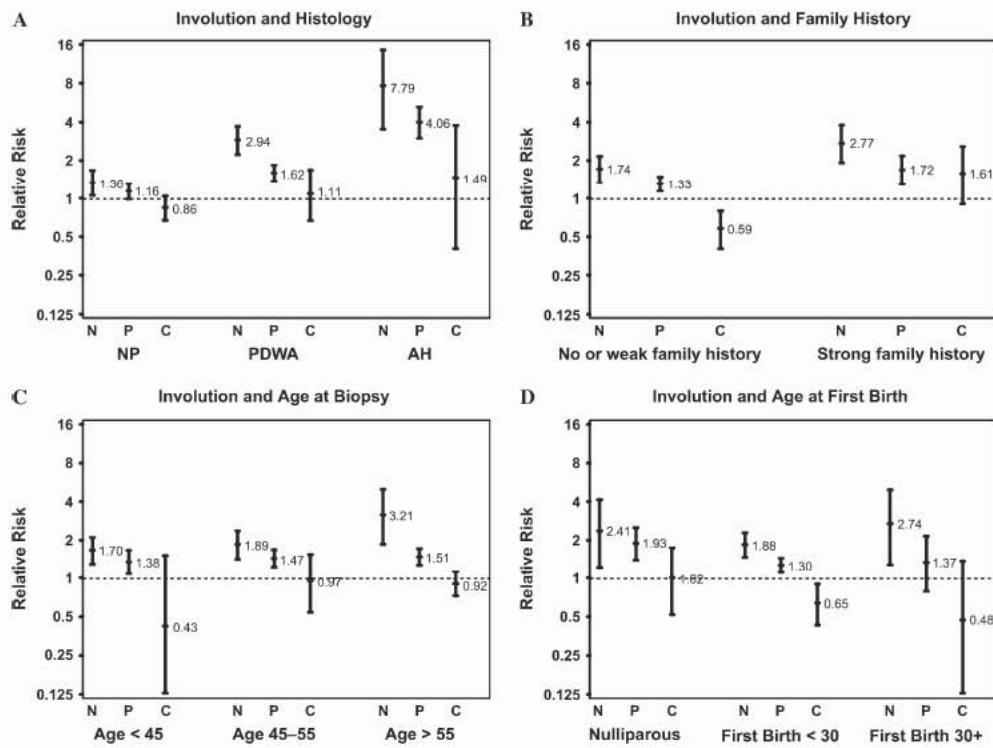
Similarly, progressive increases in lobular involution in background breast tissue was associated with reduced risk of breast cancer among women with benign proliferative disease, even those with atypia (Fig. 2). Among women with atypia, no involution was more strongly associated with a higher risk of breast cancer (RR = 7.79, 95% CI = 3.56 to 14.81) than complete involution (RR = 1.49, 95% CI = 0.41 to 3.82) or partial involution (RR =

4.06, 95% CI = 3.03 to 5.33) (test for heterogeneity,  $P = .003$ ). Among women with proliferative disease without atypia, no involution was also associated with a higher risk of breast cancer (RR = 2.94, 95% CI = 2.26 to 3.75) than complete involution (RR = 1.11, 95% CI = 0.68 to 1.71). The same pattern held true for those with nonproliferative breast disease; i.e., those with no involution had a higher risk than those with complete or partial involution.

Lobular involution modified the influence of family history on risk of breast cancer (Fig. 2). Among women with a strong family history of breast cancer, no involution was associated with the highest risk of breast cancer (RR = 2.77, 95% CI = 1.94 to 3.84), followed by partial involution (RR = 1.72, 95% CI = 1.32 to 2.20) and then by complete involution (RR = 1.61, 95% CI = 0.92 to 2.61). Among women with no or a weak family history of breast cancer and complete involution, the risk of breast cancer (RR = 0.59, 95% CI = 0.41 to 0.81) was approximately half of that for the general population, which was based on Iowa SEER data, and approximately fivefold less than the risk of those with strong family history and no involution (i.e., RR = 2.77, 95% CI = 1.94 to 3.84).

Lobular involution also modified the risk associated with parity or age at birth of the first live child. Among nulliparous women and women whose first live child was born when she was at least 30 years old, no lobular involution was associated with increased risks of breast cancer (RR = 2.41, 95% CI = 1.25 to 4.21, and RR = 2.74, 95% CI = 1.31 to 5.03, respectively). However, among these same two groups of women, when complete lobular involution had occurred, there was no increase in risk (RR = 1.02, 95% CI = 0.53 to 1.78, and RR = 0.48, 95% CI = 0.10 to 1.40, respectively). Among women whose first live child was born when she was younger than 30 years old, complete lobular involution was associated with a decreased risk of breast cancer (RR = 0.65, 95% CI = 0.44 to 0.91).

We also asked whether the era in which the biopsy examination was performed—namely, before or after widespread adoption of mammography—affected the results. In the first 15 years of the cohort (from 1967 through 1981), 78% of biopsy examinations were done because of a palpable concern (i.e., a palpable



**Fig. 2.** Association of breast cancer risk with lobular involution and other variables. Relative risks (as indicated) and their 95% confidence intervals (error bars) reflect the observed number of events compared with the number of expected events on the basis of Iowa Surveillance, Epidemiology, and End Results data. All results account for the effects of age and calendar period.

A) Involution and histology. B) Involution and family history. C) Involution and age at biopsy examination. D) Involution and age at birth of first live child. N = no involution; P = partial involution; C = complete involution; NP = nonproliferative; PDWA = proliferative disease without atypia; AH = atypical hyperplasia.

lump detected during a clinical breast examination or by the patient), and 22% were done because of an abnormal mammogram. From 1982 through 1991, 40% of the biopsy examinations were done because of a palpable concern, and 60% were done because of an abnormal mammogram. The relative risks of breast cancer by involution status and by dates (Table 3) indicated that associations between extent of involution and risk were similar in the pre- and postmammography time periods.

#### Lobular Involution: Localized or Field Effect

To address whether or not the degree of involution was relevant only to the area of the biopsy or was representative of the field of breast tissue, we examined 1) whether, for women with bilateral benign biopsy examination results, involution results were concordant and 2) whether the degree of involution at the benign biopsy site was associated with the risk of ipsilateral breast cancer or with both ipsi- and contralateral breast cancers. A subset of 245 women had bilateral biopsy examinations performed at the same time. In 203 (83%) of these women, the same category of involution (no, partial, or complete) was found in the biopsy tissue from both breasts. In 41 (17%), there was a difference of one category between the two breasts. Only one individual had complete involution in the biopsy tissue of one breast and no involution in the

contralateral sample. These results indicate a high level of agreement in involution measured across multiple biopsy specimens within a woman ( $\kappa$  coefficient = 0.72, 95% CI = 0.64 to 0.80; test for agreement beyond that expected by chance  $P < .001$ ).

We next investigated the extent of involution and the laterality of subsequent breast cancers. In our cohort overall, there is a slight predominance of ipsilateral breast cancers (55.5%) over contralateral breast cancers (44.5%), as reported previously (8); this result is thought to reflect the presence of some direct precursors among these lesions. To determine whether involution at the site of the benign breast disease was relevant to the contralateral breast, we examined the ratio of ipsilateral to contralateral events by degree of involution. With no involution, the ratio was 53.6% ipsilateral to 46.4% contralateral; for partial involution, the ratio was 55.9% to 44.1%; and for complete involution, the ratio was 53.5% to 46.5% (chi-square test for difference in percent ipsilateral across involution status,  $P = .85$ ). Thus, the relationship between involution extent and breast cancer risk was observed in both the ipsi- and contralateral breast.

#### DISCUSSION

We characterized the degree of lobular involution in the background breast tissue in a large cohort of women with benign

Table 3. Relative risk of breast cancer by involution and year of biopsy

Extent of involution and year of biopsy	No. of women	No. of person-years	No. of observed events	No. of expected events*	Relative risk (95% CI)†
No involution					
1967-1981	957	21 886	105	56.9	1.85 (1.51 to 2.23)
1982-1991	670	10 384	45	22.7	1.98 (1.44 to 2.65)
Partial involution					
1967-1981	2381	49 080	204	157.3	1.53 (1.34 to 1.73)
1982-1991	2816	41 329	200	142.8	1.40 (1.21 to 1.61)
Complete involution					
1967-1981	740	12 524	55	46.8	1.18 (0.89 to 1.53)
1982-1991	1172	15 851	51	69.7	0.73 (0.54 to 0.96)

\*Number of events expected on the basis of Iowa Surveillance, Epidemiology, and End Results breast cancer incidence data.

†All analyses account for the effects of age and calendar period. CI = confidence interval.

breast disease. Our data demonstrate a strong, inverse relationship between degree of involution and breast cancer risk. To our knowledge, this is the first study to systematically examine age-related involution in the context of breast cancer risk. Furthermore, greater degrees of involution reduced breast cancer risk even in high-risk subsets defined by age, atypia, reproductive history, or family history. There was a strong direct association between involution and increasing age. There was an inverse association between involution and parity.

As in this study, others have found that older women tend to have fewer lobules or only lobule remnants (4,5). Cowan and Herbert (4) performed a detailed autopsy study of the breast tissue from 102 women, aged 50-104 years, who died without known breast disease. Although considerable individual variability was present, they described a progressive loss of lobules with increasing age. Earlier reports state that age-related involution has already begun in women under the age of 40 years (1,5). Our data confirm that this process is present, at least to a partial degree, in many younger women.

We hypothesize that the degree of involution detected at the benign biopsy site reflects that of the overall field of a woman's breast tissue. We believe that this hypothesis is reasonable because of our results showing a similar likelihood of contralateral and ipsilateral breast cancers by involution status at the site of the benign breast disease and because of the high concordance in involution status in women who had bilateral biopsy examinations. However, our study design cannot answer this question definitively. To do so would require examination of the extent of involution throughout all of a woman's breast tissue.

It is widely appreciated that, as women age, their risk of breast cancer increases. But the rate of increase of breast cancer slows appreciably at approximately age 50 years (10,11), which has been attributed to a reduction in ovarian hormonal production (12). We observed a definite increase in the process of involution at approximately age 50 years (with complete involution present in 5.8% of women aged 40-49 years and in 21.6% of women aged 50-59 years). These data raise the possibility that involution may be contributing to the slowing in the rate of increase of breast cancer among women older than 50 years, as speculated by Henson and Tarone (7).

We examined various factors besides age for their association with degree of involution. We found an inverse association between lobular involution and parity. Others have also reported that the more children a woman has, the more likely she is to have persistence of lobular structures (1,5), which we found was associated with increased risk of breast cancer. Yet, multiparity is

generally considered to reduce the risk of breast cancer (13,14). Several factors may explain this apparent contradiction. First, we do not have data on the age at each child's birth for the women in our cohort. Some epidemiologic work has suggested that full-term pregnancies after 35 years of age are associated with an increased risk of breast cancer (7,15). Thus, data on a woman's age at each pregnancy and on her age at breast biopsy examination would help to evaluate more definitively the relationships of parity, involution, and breast cancer risk. In addition, our study was limited by the relatively large size of the group of women categorized as having partial involution. More specific, quantitative measures of degree of involution should be explored to determine whether the association between parity status and degree of involution can be defined more precisely. Given the inverse association between complete involution and multiparity and given that both are associated with reduced risk of breast cancer, we hypothesize that the breast cancer risk modification associated with parity is independent of involution status.

There are several biologic mechanisms by which involution or lack thereof could alter a patient's breast cancer risk. The decrement in epithelial cell number that accompanies involution may decrease breast cancer risk simply because there are fewer epithelial cells to undergo malignant transformation. Another possibility is that aberrant involution may be a marker or phenotype reflecting underlying constitutional susceptibility for breast cancer that is present in the epithelial or stromal compartment or in their relationship with each other. Yet another possibility is that failure to undergo timely or appropriate involution allows prolonged exposure of epithelial cells to intrinsic and/or extrinsic mutagenic stresses (16-20). In this model, the prime targets of such mutagenic processes, such as stem cells or early progenitors, may become quiescent during the process of involution. Experiments to characterize the epithelial and stromal mediators present in tissue with and without involution, in women with and without subsequent cancer, should help to clarify the mechanism of risk reduction.

For our work to date, we divided extent of involution into three categories. We recognized that, although the morphologic patterns of age-related lobular involution have been defined (1,4,5), no histologic standard exists for evaluating the extent of breast involution. In particular, there is no well-characterized method for grading partial degrees of involution. For this reason, we attempted to classify degree of involution with the least amount of subjectivity. Thus, by deciding only whether breast tissue had no lobular involution versus almost complete involution and then by combining the remainder into one category of partial involution, we minimized the subjectivity inherent in judging percent involution.

Our study has several limitations. First, these findings do not necessarily pertain to all women because the cohort studied included women who had a breast biopsy because of some concern. Nevertheless, women with benign breast disease make up a large population who are understandably concerned about their breast cancer risk (estimated at 1 million US women each year) (21–23). Another limitation lies in our current very broad category of partial involution. This category encompasses a wide range of involution extent (1%–74% of lobules involuted). We expect that more specific gradations would support more refined association studies. Finally, we did not have complete risk factor data for all the women in the cohort, largely because the women with biopsy examinations in the earlier years of the cohort are now elderly or deceased. Fortunately, for purposes of this report, we did not have to depend on the questionnaire for involution status or for cancer outcomes (which were available from our comprehensive Mayo medical record). We had completed questionnaires for 63.8% of the patients with breast cancer and 61.6% of the patients without breast cancer in the cohort.

There are other approaches to the study of involution and breast cancer risk. Henson and Tarone (7) suggested an autopsy case–control series to look at involution as a possible risk factor for breast cancer. Although this approach would provide access to extensive amounts of breast tissue, the availability of clinical risk factor information and of a sufficient number of subjects could be limiting. Other women who have breast tissue removed in the course of clinical care are those who have reduction mammoplasty or prophylactic mastectomy. These women are, respectively, those who have breast hypertrophy or a hereditary predisposition to breast cancer. Although involution (or lack thereof) in these women is of considerable interest, their tissue is not necessarily representative of the general population.

The mechanisms controlling age-related involution are of considerable interest. Molecular programs that control postlactational involution in rodents have been studied extensively (24). With postlactational involution, there is dramatic reversal of the developmental changes wrought by pregnancy. Specifically, there is widespread apoptosis of alveolar epithelial cells followed by removal of apoptotic debris and remodeling of the stroma and extracellular matrix (24). These events occur within a matter of days of abrupt weaning and restore the gland to its prepregnancy state. In contrast, the molecular orchestration of age-related involution, to our knowledge, has not yet been characterized.

In the past, for women with benign breast biopsy results, the type and extent of epithelial proliferation present in their biopsy has been the principal way to stratify their risk. Results of our study indicate that assessing the status of lobular involution in the biopsied tissue may ultimately add to risk prediction capabilities. It is notable, as shown in Fig. 2, C, that some of the most extreme risk estimates are observed in women whose involution status is unusual for their age—namely, young women with complete involution (RR = 0.43, 95% CI = 0.13 to 1.55) and women older than 55 years with no involution (RR = 3.2, 95% CI = 1.90 to 5.08). It is tempting to speculate that the process of complete involution may be protective and, conversely, that lack of involution identifies higher risk groups. However, confidence intervals were wide around the estimates for these less common categories.

In summary, we have evaluated the extent and effect of age-related lobular involution in a cohort of approximately 9000

women who had a benign breast biopsy examination. We observed a statistically significant reduction in risk of breast cancer among those women whose breast tissue had undergone extensive lobular involution, which was apparently independent of other markers of risk. Among women with benign breast disease, assessment of extent of involution may help to fine-tune current risk prediction approaches. Elucidation of the mechanism of lobular involution may reveal ways to promote the process as a means of risk reduction.

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#### NOTES

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## EDITORIALS

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### Lobular Involution: the Physiological Prevention of Breast Cancer

Donald Earl Henson, Robert E. Tarone, Hala Nsouli

It truly is a remarkable event when traditional pathologic observations lead to new ideas about the prevention of cancer. In this issue of the Journal, Milanese et al. (1), through a histologic review of breast biopsy specimens, show that the extent of age-related lobular involution is strongly associated with a reduced risk of breast cancer. Breast cancer risk decreased with increasing extent of involution in both high-risk and low-risk subgroups defined by age, epithelial atypia, reproductive history, and family history of breast cancer.

Beginning in the premenopausal period, lobular involution is a physiologic process that occurs over many years whereby the parenchymal elements in the breast progressively atrophy and disappear (2,3). The study reported by Milanese et al. represents a unique application of the Mayo Benign Breast Disease Cohort to investigate prospectively involution as a risk factor for breast cancer. It is the first study, to our knowledge, to substantiate a hypothesis that is based on pathologic and epidemiologic considerations that delayed involution is a major risk factor for breast cancer (4,5).

As for an explanation of the effect of lobular involution on breast cancer risk, it has been suggested that a reduction in mammary gland tissue that results from involution should lead to a reduction in breast cancer because a progressively smaller amount of epithelial tissue is available for malignant transformation (1,5). The result of involution, therefore, can be considered physiologically analogous to a partial prophylactic mastectomy, with a corresponding reduction in breast cancer risk.

Although a reduction in mammary tissue is a plausible explanation, the underlying issue is one of aging or, more precisely, the failure of breast tissue to age normally. The aging process in the breast is under control of various hormones and does not follow the pattern seen in other organs or tissues. Pathologists have long commented on the possibility that persistent atypical lobules might be precursors of invasive breast cancer (6,7). It seems paradoxical that an organ that normally undergoes complete or near complete physiologic atrophy would be a site in which cancer rates steadily increase with age. The continuing increase in breast cancer risk with age is likely associated with the persistence of glandular epithelium beyond the time of normal involution, reflecting an abnormal delay of the aging process in the breast (4,5).

Except for morphologic observations concerning age of onset and progression with age, practically nothing is known about the process of involution. Even less is known about factors that control involution or that delay or accelerate the process. In this context, it is unknown whether the rate of involution is genetically determined and whether known breast cancer risk or environmental factors alter the rate of involution.

Evidence indicates that some risk factors for breast cancer may interfere or affect the process of involution. In the Mayo study, women with benign proliferative breast disease were substantially less likely to have complete involution than were

women with benign nonproliferative disease, and women with a strong family history of breast cancer had slightly less advanced involution than women without such history (1). Late age of menopause, which increases the risk of breast cancer, is likely to result in delayed involution because of persistence of estrogen activity (8). Women whose first full-term pregnancy occurs after age 35 years have an elevated risk for breast cancer compared with nulliparous women or with women whose first pregnancy was at a much younger age (9,10). After the commencement of involution, late pregnancy with its concomitant increase in the proliferation of the ductal-alveolar epithelium is likely to interrupt the normal process of involution, which typically begins between 30 and 40 years of age. Oophorectomy, which protects against breast cancer (11), leads to the same type of atrophy of breast parenchyma in young women as that seen in older women (12). The reduction in risk may be due to the acceleration of involution induced by oophorectomy.

One of the most striking findings in the study of Milanese et al., however, is the degree to which the strong association between extent of involution and breast cancer risk was independent of all known breast cancer risk factors that were investigated (1). This observation suggests that factors unrelated to known risk factors are responsible for the protective effect of involution. For this reason, a greater understanding of the biologic basis for involution will be required to elucidate the mechanisms of the protective effect of lobular involution on breast cancer risk.

The observations reported by the Mayo group may find practical applications for risk prediction (1). It may be useful for pathologists to report the extent of involution in addition to any epithelial changes found in breast biopsy specimens that do not contain cancer. It will be important to determine the extent to which mammographic breast density serves as a surrogate for the extent of involution. By taking extent of involution into account, it should be possible to increase the predictive ability of breast cancer risk models.

Results of the Mayo study provide a new paradigm for breast cancer research and prevention. Age has always seemed the opponent because of the increasing risk of breast cancer with age,

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but age may now become an ally. The challenge will be to unravel the natural history of involution and the normal process of aging in the breast. Eventually, involution could become a useful surrogate endpoint for research in breast cancer prevention. A possible approach to prevention may be to develop strategies that achieve complete involution as early as possible after childbearing is completed.

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## Stratification of Breast Cancer Risk in Women With Atypia: A Mayo Cohort Study

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### ABSTRACT

#### Purpose

Atypical hyperplasia is a well-recognized risk factor for breast cancer, conveying an approximately four-fold increased risk. Data regarding long-term absolute risk and factors for risk stratification are needed.

#### Patients and Methods

Women with atypical hyperplasia in the Mayo Benign Breast Disease Cohort were identified through pathology review. Subsequent breast cancers were identified via medical records and a questionnaire. Relative risks (RRs) were estimated using standardized incidence ratios, comparing the observed number of breast cancers with those expected based on Iowa Surveillance, Epidemiology, and End Results (SEER) data. Age, histologic factors, and family history were evaluated as risk modifiers. Plots of cumulative breast cancer incidence provided estimates of risk over time.

#### Results

With mean follow-up of 13.7 years, 66 breast cancers (19.9%) occurred among 331 women with atypia. RR of breast cancer with atypia was 3.88 (95% CI, 3.00 to 4.94). Marked elevations in risk were seen with multifocal atypia (eg, three or more foci with calcifications [RR, 10.35; 95% CI, 6.13 to 16.4]). RR was higher for younger women (< 45; RR, 6.76; 95% CI, 3.24 to 12.4). Risk was similar for atypical ductal and atypical lobular hyperplasia, and family history added no significant risk. Breast cancer risk remained elevated over 20 years, and the cumulative incidence approached 35% at 30 years.

#### Conclusion

Among women with atypical hyperplasia, multiple foci of atypia and the presence of histologic calcifications may indicate "very high risk" status (> 50% risk at 20 years). A positive family history does not further increase risk in women with atypia.

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### INTRODUCTION

Atypical hyperplasia is a well-established risk factor for subsequent breast cancer. Multiple studies corroborate an approximately four-fold increased risk of breast cancer in women undergoing surgical biopsy with a finding of atypia.<sup>1-7</sup> Despite good concordance on the estimated relative risk (RR) with atypia, estimates of absolute risk with long-term follow-up are not well established. Reliable breast cancer risk estimates for women with atypia are crucial for risk-benefit analysis and decision making regarding risk-reduction strategies.

The Gail model in current use predicts a dramatically increased risk for those women who have both atypia and a family history (over that of atypia alone).<sup>8</sup> Prior published literature has stated that the

risk of breast cancer abates considerably after 10 years after a diagnosis of atypia,<sup>9</sup> whereas more recent evidence indicates otherwise.<sup>10</sup> It is also unclear whether breast cancer risk is higher in cases of atypical ductal hyperplasia (ADH) versus atypical lobular hyperplasia (ALH).

Here, we present a comprehensive description of breast cancer risk in women with atypical hyperplasia, based on 331 women with atypia in the Mayo Benign Breast Disease Cohort. Our investigation addresses the effect of family history on atypia risk, the effect of time since biopsy, the influence of ductal versus lobular histology, effects of age at atypia diagnosis, and presence of calcifications on breast cancer risk. In addition, we provide absolute risk estimates over time, and we also present a new histologic feature of atypia—multifocality—that stratifies breast cancer risk among women with atypia.

## PATIENTS AND METHODS

**Study Population**

Entry criteria for the study cohort have been previously described.<sup>1</sup> Briefly, this comprises an institutional review board-approved study of women ages 18 to 85 years who had a benign breast biopsy via surgical excision during 1967 to 1991. The initial cohort included 9,087 women.<sup>1</sup> With additional follow-up, data are now available for 9,376 women, 331 (3.5%) of whom had atypical hyperplasia.

**Follow-Up**

Follow-up for breast cancer events (including both invasive cancer and ductal carcinoma in situ [DCIS]) and risk-factor information were obtained for all women with atypia through the Mayo medical record and a study questionnaire. Family history was classified as negative, strong, or weak. The criteria for a strong family history were at least one first-degree relative with breast cancer before the age of 50 years or two or more relatives with breast cancer, with at least one being a first-degree relative. Any lesser degree of family history was considered weak.<sup>1</sup>

**Histology**

All available archival hematoxylin and eosin-stained sections were evaluated by our breast pathologist (D.W.V.), without knowledge of the original histologic diagnoses or patient outcomes. The number of slides reviewed per case was variable because of the retrospective nature of the study, with a mean of 3.2 (standard deviation, 3.7). Calcifications were recorded for each case when seen histologically. A diagnosis of ADH or ALH was based on the criteria of Page et al.<sup>2,11</sup> ADH was characterized by filling and distension of involved ducts by an architecturally complex proliferation of monotonous cells forming "punched out" (cubiform-like) secondary lumens or micropapillary formations. Although well-developed examples of ADH share some morphologic features with low-grade DCIS, the latter is characterized by tumefactive growth (requiring complete involvement of >2 contiguous lumens) as well as greater nuclear enlargement and hyperchromatism. For each example of atypical hyperplasia, the number of separate foci was defined. Multifocal atypia required its identification in more than one terminal duct lobular unit (TDLU) as defined by clear separation from another by nonspecialized interlobular mammary stroma. All cases of multifocal atypia were agreed on by two study pathologists (D.W.V. and C.A.R.).

The primary study pathologist (D.W.V.) identified 332 cases of atypia from the entire benign breast disease cohort of 9,376. To address concerns of reproducibility in the diagnosis of atypia,<sup>12</sup> we performed a nested study of concordance, blinding another pathologist (H.B.) to the study diagnoses in a random subset of several hundred samples from the original cohort, including nonproliferative lesions, proliferative disease without atypia, and atypical hyperplasia. Of 189 atypia samples reviewed for concordance, 165 (87.3%) atypias were similarly classified by subsequent independent review. Of the remaining 24 cases with differing interpretation, 18 were then judged to have atypia by joint review (D.W.V. and H.B.), and five of six remaining cases had atypia by review of a third "tiebreaker" breast pathologist (C.A.R.). The one case in question was excluded from further analysis, leaving a total of 331 subjects for study.

**Statistical Analysis**

Follow-up was defined as the number of days from benign biopsy to date of breast cancer diagnosis, death, or last contact. We estimated RRs with standardized incidence ratios (SIRs) and 95% CIs, dividing observed numbers of incident breast cancers by expected counts. We calculated expected counts by apportioning each individual's follow-up time into 5-year age and calendar-period categories, and applying these person-years to population-based incidence rates, thereby accounting for differences in these variables. We used the Iowa Surveillance, Epidemiology, and End Results (SEER) registry as the reference population, because of its proximity to the Mayo Clinic catchment area and racial similarities compared with our cohort.<sup>1</sup> We extrapolated incidence-rate data for cohort follow-up occurring outside the SEER time-frame (1973-2002), such that person-years before 1973 were applied to 1973 to 1975 incidence rates, and person-years subsequent to 2002 were applied to

2001 to 2002 incidence rates. Assuming a two-sided test of hypothesis and a type I error rate of 0.05, we would have 80% power to detect SIRs as low as 3.61 if the expected event count is 2.5, as low as 2.97 if the count is 4.2, as low as 2.08 if the count is 10.3, and as low as 1.84 if the count is 17. Note that these expected counts reflect the approximate expected numbers of events in our cohort for women with three or more foci of atypia, two foci, one focus, and all subsets combined, respectively.

Recognizing that other biologic mechanisms may modify the association of atypia and breast cancer risk, we formally assessed the potential differential effects of these mechanisms using Poisson regression analyses. This approach allowed us to estimate SIRs with the flexibility that generalized linear models provide, such as covariate adjustment and tests for trend or heterogeneity across subgroups. For all analyses, the log-transformed expected event rate for each individual was modeled as the offset term.

We displayed observed and expected event rates using cumulative incidence curves and corresponding 95% confidence limits, accounting for the effects of death as a competing risk.<sup>13</sup> Expected events were calculated for each 1-year follow-up interval in a manner similar to that used for determining SIRs. A modified Kaplan-Meier approach was used to cumulate expected incidence over these intervals. The expected curve was then smoothed using linear interpolation.

We compared the RR of ipsilateral versus contralateral breast cancer overall and across different medical characteristics using ratios of corresponding incidence rates. When calculating incidence for ipsilateral cancer, individuals with contralateral cancer were excluded at their diagnosis date, and vice versa. Women with missing laterality, or having bilateral biopsies or cancer, were excluded for both events. The RRs are equivalent to ratios of observed events, as the approach yields identical person-years for each event type. We thus used properties of the binomial distribution to obtain exact 95% CIs for these RRs.<sup>14</sup> All statistical tests were postulated a priori and were two-sided, and all analyses were conducted using the SAS software system (SAS Institute, Cary, NC).

## RESULTS

**Characteristics of Patients and Pathologic Specimens**

A total of 331 women with atypia were identified in our cohort between 1967 and 1991. In Table 1, we present the patients' vital status, breast cancer status, family history, age at biopsy, year of biopsy, indication for biopsy, and histologic features. Women were likely to be older than 55 at diagnosis of atypia (55.9%), and 42.9% had a family history of breast cancer (23.3% with a strong family history). Histologic findings included calcifications in most cases of atypia (68.6%); most cases (60.1%) had only one focus of atypia. The relative percentages of women with one, two, and three or more foci of atypia remained stable over the time period of the cohort. The proportions of women with ADH and ALH were similar.

**Subsequent Breast Cancer Risk and Modifying Factors**

The 331 women with atypia were followed for a total of 4,543 person-years (mean 13.7 years), with 66 (19.9%) observed breast cancer events to date. The histologic types are known in 61 of these, with 53 (86.9%) of 61 invasive cancers and eight (13.1%) of 61 DCIS. The majority of invasive cancers were ductal type (47 of 53, 89%), and the remaining six invasive lobular cancers were divided between the ALH and ADH subgroups. Table 2 shows the estimated RRs for breast cancer associated with various characteristics. The overall group with atypia demonstrates a four-fold RR of breast cancer (RR, 3.88; 95% CI, 3.00 to 4.94) compared with the general population.

**Family History**

There were no significant differences in RR seen among the subgroups with a strong family history (RR, 3.59; 95% CI, 1.96 to

Characteristic	No.	%
Vital status		
Deceased	101	30.5
Alive	230	69.5
Breast cancer status		
Noncase	265	80.1
Case	66	19.9
Age at biopsy, years		
Mean	58	
SD	12	
Family history of breast cancer		
Unknown	42	
None	185	57.1
Weak	56	19.4
Strong	88	23.5
Age at BBD, years		
≤ 45 at BBD Dx	48	13.9
46-55 at BBD Dx	100	30.2
≥ 56 at BBD Dx	183	55.9
Year of BBD		
1967-1971	15	4.5
1972-1976	35	10.6
1977-1981	40	12.1
1982-1986	96	29.0
1987-1991	145	43.9
Indication for biopsy		
Unknown	8	
Palpable mass	139	42.8
Mammographic abnormality	188	57.2
Calcifications		
Without calcifications	104	31.4
With calcifications	227	68.6
Histologic subtype		
Lobular	176	52.9
Ductal	142	42.9
Lobular and ductal	14	4.2
No. of foci of atypia		
1	198	60.1
2	81	24.5
≥ 3	51	15.4

Abbreviations: SD, standard deviation; BBD, benign breast disease; Dx, diagnosis.

6.03), a weak family history (RR, 5.59; 95% CI, 3.20 to 9.09), or a negative family history (RR, 3.81; 95% CI, 2.60 to 5.37; Table 2; Fig 1A).

#### Age at Biopsy

Women with atypia diagnosed at younger age had a higher RR compared with age-matched expected rates (Table 2; Fig 1C). The RR was 6.76 at age less than 45, 5.10 at age 45 to 55, and 2.87 at age greater than 55 years ( $P$  for trend = .01). The increased risk seen in younger women was not due to a positive family history, because there was no difference in risk for women with and without a family history in each age subgroup (data not shown).

#### Number of Foci of Atypia

Increasing risk was seen with increasing foci of atypia: RR = 2.33 with a single focus, 5.26 for two foci, and 7.97 for three or more foci.

with a highly significant test for trend ( $P$  < .001; Fig 1B). The increased risk seen with multiple foci of atypia was not due to predominance of young (higher risk) women in those subgroups; women younger than 45 years constituted only 4.94% and 7.84% of the subgroups with two and three foci of atypia, compared with 19.1% of the subgroup with one focus. Multivariate Poisson regression analysis also confirmed that young age and multifocality contributed independently to increased risk.

#### Calcifications

Risk was dramatically increased in the small group of women ( $n = 38$ ) with both calcifications and three or more foci of atypia (RR, 10.4; 95% CI, 6.13 to 16.4). However, women with calcifications and less than three foci of atypia (RR, 3.1) had risk similar to that of patients with fewer than three foci of atypia and no calcifications (RR, 3.31).

#### Histologic Type of Atypia

Histologic type of atypia did not affect breast cancer risk, because the RR of breast cancer was the same for ADH and ALH, although the few individuals with both histologic types may have higher risk (Fig 1D).

#### Indication for Biopsy

Breast cancer risk was similar whether a palpable or mammographic concern prompted the biopsy.

#### At-Risk Time Interval and Cumulative Incidence of Breast Cancer

The RR of breast cancer for the entire group with atypical hyperplasia was elevated persistently beyond 15 years, with a 20-year cumulative risk of 21% (95% CI, 14% to 28%) and a 25-year cumulative risk of 29% (95% CI, 20% to 38%; Fig 2). Stratification based on number of foci of atypia demonstrates a cumulative incidence of 18% for a single focus, 45% for two foci, and 48% for three or more foci of atypia at 25 years of follow-up (Fig 3).

#### Laterality of Breast Cancer Risk

Of the 66 women with atypia who subsequently developed breast cancer, side of cancer and side of atypia are known in 57 cases. Although cancer was more frequent in the ipsilateral breast, this difference was not statistically significant for the overall group with atypia (RR, 1.38 for ipsilateral v contralateral event; 95% CI, 0.79 to 2.21). However, the 32 women with atypia who developed breast cancer within 10 years of their benign biopsy were 2.2 times more likely (95% CI, 1.02 to 4.86;  $P = .05$ ) to develop cancer in the same breast versus the opposite breast. Women with ADH had higher ipsilateral risk (RR, 1.50; 95% CI, 0.62 to 3.82), and women with three or more foci also had higher risk of ipsilateral breast cancer (RR, 2.20; 95% CI, 0.71 to 4.52), although these increases did not reach statistical significance due in part to small numbers of events and modest statistical power for these analyses. Women with ALH had similar cancer risk in both breasts (RR, 1.08; 95% CI, 0.45 to 2.14).

#### DISCUSSION

Having reliable breast cancer risk estimates for women with atypical hyperplasia is imperative in order to tailor their care appropriately. For women with atypia, the Gail model is the only model available for risk

Table 2. Risk Factors for Breast Cancer Among the 331 Women With Atypia From the Mayo Benign Breast Disease Cohort Study

Variable	No.	Person-Years	Observed Events	Expected Events	RR	95% CI
Overall atypia group	331	4,543	68	170	3.88	3.00 to 4.94
Age at benign biopsy, years						
< 45	46	678	10	1.5	6.76	3.24 to 12.40
45-55	100	1,540	28	5.1	5.10	3.33 to 7.48
> 55	185	2,325	30	10.4	2.87	1.98 to 4.10
No. of foci of atypia						
1	159	2,792	24	10.3	2.33	1.49 to 3.48
2	81	1,056	22	4.2	5.26	3.39 to 7.96
≥ 3	51	655	20	2.5	7.97	4.97 to 12.30
Calcifications						
Without	104	1,529	18	5.5	3.21	1.90 to 5.08
With	227	3,013	48	11.4	4.71	3.10 to 5.58
< 3 foci	189	2,538	30	9.7	3.10	2.09 to 4.43
≥ 3 foci	38	478	18	1.7	10.4	6.13 to 16.40
Histologic subtype						
Lobular	176	2,635	34	9.3	3.67	2.54 to 5.13
Ductal	142	1,815	27	7.0	3.83	2.63 to 5.58
Lobular and ductal	14	194	5	0.7	7.10	2.31 to 16.5
Family history of breast cancer						
None	185	2,226	32	8.4	3.81	2.60 to 5.37
Weak	66	763	16	2.9	5.59	3.20 to 9.09
Strong	68	1,029	14	3.9	3.59	1.96 to 6.03
Indication for biopsy						
Palpable mass	129	2,068	33	7.2	4.55	3.13 to 6.39
Mammographic abnormality	166	2,409	32	9.5	3.36	2.30 to 4.74

NOTE. RR and CI represent standardized incidence ratio and 95% confidence limits, comparing observed number of events to those expected based on Iowa Surveillance, Epidemiology, and End Results data. All results account for the effects of age and calendar period.

Abbreviation: RR, relative risks.

prediction.<sup>8</sup> In this model, calculations of risk for women with atypia and a family history are dramatically higher, based on prior evidence from the Nashville study.<sup>2</sup> Therefore, for a 50-year-old

white woman with menarche at age 12, first birth at 24, and atypia on breast biopsy, the predicted lifetime risk of breast cancer is 17.5%. If that same woman also has a first-degree relative with

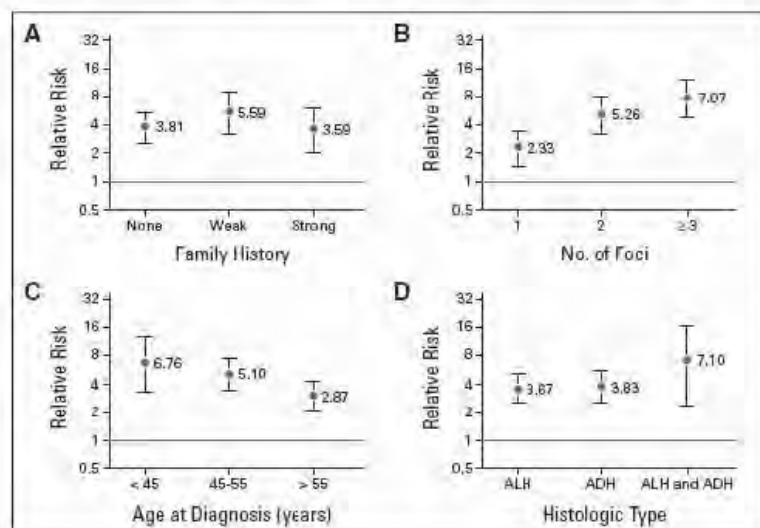
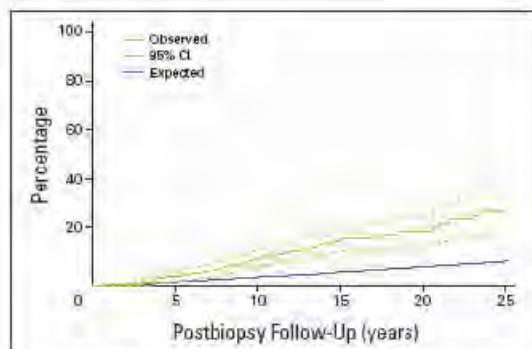


Fig 1. Stratification of breast cancer risk for women with atypical hyperplasia. (A) Family history, (B) number of foci, (C) age at diagnosis, and (D) histologic type. Relative risks expressed as standardized incidence ratios and 95% confidence limits, comparing observed number of events to those expected based on Iowa Surveillance, Epidemiology, and End Results data. All results account for the effects of age and calendar period. Horizontal lines at 1.0 reflects the overall expected relative risk in the cohort. ALH, atypical lobular hyperplasia; ADH, atypical ductal hyperplasia.



**Fig 2.** Cumulative risk of breast cancer over time. Observed cumulative breast cancer incidence among women with atypical hyperplasia, with 95% CIs represented by stippled lines. Expected breast cancer events were calculated by applying age- and calendar period-stratified person-years of observation to corresponding Iowa Surveillance, Epidemiology, and End Results breast cancer incidence rates. Observed and expected events cumulated after accounting for death as a competing risk.

breast cancer her lifetime risk doubles to 34%. Our data indicate that the Gail model predicts inaccurately for such women because the increased risk of breast cancer associated with atypia is independent of the effect of family history.

Women in our cohort with atypia and a positive family history of breast cancer had no additional increased risk of breast cancer over that of atypia alone. This finding counters the commonly held view proposed by the Nashville study (ie, that atypia and a positive family history increase breast cancer risk additively). When data from other major studies of benign breast disease are considered along with the Mayo findings, the preponderance of evidence calls into question the result from the Nashville group. In that study, the subgroup of women with atypia and a family history was small ( $n = 39$ ) with an RR of 8.9 (95% CI, 4.8 to 17), compared with 3.5 (95% CI, 2.3 to 5.5) in 193

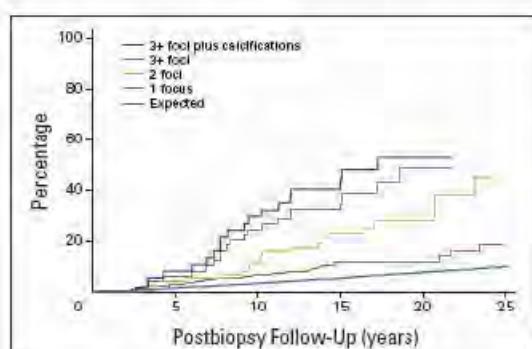
women with atypia and no family history.<sup>2</sup> In contrast evaluation of a much larger population in the Breast Cancer Detection and Demonstration Project showed similar frequencies of breast cancer in women with atypia and family history (16 of 261, 6.1%) compared with those with atypia alone (51 of 1,044, 4.9%).<sup>4</sup> Recent data from the Nurses' Health Study confirm our finding that a family history of breast cancer in a first-degree relative does not further increase risk among women with atypical hyperplasia.<sup>12</sup> To explain these findings, we postulate that atypical hyperplasia is a phenotype reflecting increased risk; this phenotype derives from both inherited risk and lifetime exposures. Thus, the histologic presence of atypia already reflects the increased breast cancer risk inherent in a positive family history.

We have identified a new histologic variable that appears to stratify risk in women with atypia: multifocality. The RR of breast cancer increases in a dose-response fashion for women with one, two, and three or more foci of atypia, with a statistically significant test for trend. With a single focus, the cumulative incidence of breast cancer reached 18% at 25 years. For women with two or more foci of atypia, the cumulative risk of breast cancer was greater than 40% at 25 years. Moreover, in the highest risk subgroup of women with three or more foci and histologic calcifications, the cumulative incidence exceeded 50% over 25 years. This level of risk approaches that reported for carriers of BRCA mutations.<sup>16</sup> In line with our observation, differential risk based on extent of disease has been established for lobular neoplasia (ie, ALH v lobular carcinoma),<sup>17</sup> and the number of foci of atypia found in core needle biopsy specimens correlates with the likelihood of finding cancer at surgical excision.<sup>18</sup>

Some may question whether multifocal atypias may actually represent subtle *in situ* carcinoma, particularly those of the ADH type. In cases of multifocal ADH, it should be emphasized that individual foci arose in separate and distinct terminal duct lobular units, none of which measured more than 2 mm. Hence, these examples failed to exhibit the confluent degree of cellular proliferation requisite for a diagnosis of DCIS. We submit that more widespread distribution of atypical foci within breast tissue signals a larger burden of at-risk tissue that has progressed along the continuum toward breast cancer. The data presented in this article provide evidence that the extent of premalignant breast change is related to subsequent cancer risk. Since this is the first report of the clinical relevance of this histologic finding, we recognize the need for validation and plan to evaluate this factor in a more recent cohort from our institution. Furthermore, we hope that other research groups with large numbers of patients with atypical hyperplasia will also examine the relevance of multifocal atypia in their study sets.

Age at the diagnosis of atypia also emerged as a significant modifier of subsequent breast cancer risk, with a higher RR in younger women. The Nurses Health Study<sup>6</sup> and the Breast Cancer Detection and Demonstration Project<sup>4</sup> have also shown higher risk in younger women with atypia. In our cohort, this increased risk in younger women is not explained by more frequent multifocal disease or a positive family history. Perhaps atypical hyperplasia present at a young age is the result of previous oncogenic events; alternatively, breast tissue with atypia may be unusually susceptible to proposed oncogenic estrogen metabolites associated with the premenopausal hormonal environment.<sup>19</sup>

When counseling women with atypical hyperplasia, the length of time at risk is a key element in planning risk-reduction strategies. Dupont and Page<sup>2</sup> reported that the greatest risk of breast cancer after



**Fig 3.** Observed and expected cumulative breast cancer incidence among women with atypical hyperplasia, stratified by number of foci of atypia and histologic presence of calcifications. Expected events calculated by applying age- and calendar period-stratified person-years of observation among all women with atypia to corresponding Iowa Surveillance, Epidemiology, and End Results breast cancer incidence rates. Observed and expected events cumulated after accounting for death as a competing risk.

a diagnosis of atypia lies in the first 10 years, with subsequent RR reduced by half ( $P = .06$ ). By contrast, the Nurses Health Study<sup>10</sup> found that risk does not decrease over time, with RR slightly higher more than 10 years after biopsy (RR, 3.6) compared with the first 10 years (RR, 3.2). Our data confirm that the RR for breast cancer after a biopsy demonstrating atypia remains significantly elevated for at least 15 years.

Data on long-term absolute risk are more useful than RR estimates when counseling patients. Our study provides estimates of absolute risk for women with atypia and indicates a higher cumulative incidence of breast cancer with long-term follow-up than has been reported by other studies. Figures from the study of Dupont and Page show a cumulative breast cancer incidence of 13% at 20 years and 23% at 25 years in women with atypia.<sup>4</sup> The cumulative incidences identified in our cohort were higher: 21% at 20 years and 29% at 25 years. One factor contributing to this difference is our inclusion of DCIS as a recordable breast cancer event, whereas the Nashville study counted only cases of invasive breast cancer.<sup>2</sup> Because DCIS currently receives local treatment (and in some cases, systemic treatment) similar to that for early-stage invasive breast cancer, it is reasonable to include cases of DCIS when estimating risk.

Our data on the laterality of subsequent breast cancer do not allow conclusions regarding atypical hyperplasia acting as a precursor lesion, yet there is a suggestion of predilection for the ipsilateral breast that requires ongoing study. Breast cancers occurring in the first 10 years after atypia diagnosis were significantly more likely to occur in the ipsilateral breast. A recent study of gene expression profiling identified remarkably similar alterations in gene expression among ADH, DCIS, and invasive cancers found in the same specimen, supporting the role of atypical hyperplasia as a precursor lesion.<sup>20</sup> Regarding differences in ipsilateral risk for ductal versus lobular atypia, we found that risk was equal for both breasts after a diagnosis of ALH, which is consistent with the distribution of invasive breast cancers after a diagnosis of lobular carcinoma in situ.<sup>21</sup> In contrast, ADH was more likely associated with a later ipsilateral breast cancer, as has been shown for DCIS untreated after diagnostic biopsy.<sup>22</sup>

In conclusion, our study provides a comprehensive analysis of breast cancer risk associated with atypical hyperplasia. These findings

confirm a four-fold RR of subsequent breast cancer in women with atypical hyperplasia. We estimate that the long-term absolute risk of subsequent breast cancer (in situ or invasive) is higher than previously reported—at least 25% at 25 years, and as high as 50% to 60% in a high-risk subgroup defined by multifocality and calcifications. A positive family history does not confer significantly increased risk in women with atypia. Improved risk prediction and stratification is now possible to guide risk-reduction counseling for women with atypical hyperplasia.

#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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## Breast cancer risk in women with radial scars in benign breast biopsies

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**Abstract** *Background:* The risk for subsequent breast cancer in women diagnosed with radial scar lesions (RS) on benign breast biopsy remains controversial. We studied the relative risk of radial scar lesions in a large cohort of

patients with benign breast disease (BBD). *Methods:* Radial scars were identified in a BBD cohort of 9,262 patients biopsied at Mayo Clinic between 1967 and 1991. Radial scar lesions were classified as proliferative disease without atypia (PDWA) unless atypia was present (classified as atypical hyperplasia [AH]). The observed number of breast cancers developing among those with RS was compared to that expected in the general population using standardized incidence ratios (SIRs, mean follow-up interval 17 years). *Results:* RS were identified in 439 (4.7%) of the cohort members; 382 (87.0%) contained one RS, 42 (9.6%) contained two, 9 (2.0%) contained three, and 6 (1.4%) contained four or more. The majority of RS (356, 82.4%) were less than 5.0 mm in diameter; 60 (13.9%) were 5.0–9.9 mm, and 16 (3.7%) were 10.0 mm or greater. The relative risk for women with PDWA and RS was 1.88 (95% CI, 1.36–2.53), no different than PDWA without RS [relative risk 1.57 (95% CI, 1.37–1.79) ( $P = 0.29$ )]. Women with atypical hyperplasia and RS ( $n = 60$ ) had a relative risk of 2.81 (95% CI, 1.29–5.35), while those with atypia but without RS had a relative risk of 3.97 (95% CI, 2.99–5.19). *Conclusions:* RS imparts no increased breast cancer risk above that of PDWA or AH without RS.

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**Keywords** Radial scar · Breast scar

### Introduction

Radial scars (RS) are benign breast lesions of uncertain etiology and behavior. Although named as such in 1980 [1], radial scars were previously known by a variety of names, including sclerosing papillary proliferation, non-encapsulated sclerosing lesion, and infiltrating epitheliosis. As these names suggest, radial scars have a characteristic

low power stellate architecture; cystically dilated glands encircle the periphery, sometimes referred to as a 'flower head' pattern. The mostly acellular core is comprised of connective tissue and elastin, surrounded by radiating bands of compressed ducts and lobules that demonstrate dual myoepithelial and epithelial layers. Proliferative epithelial lesions are often seen within radial scars including sclerosing adenosis, hyperplasia, and papillomas [2].

The growth pattern in RS can resemble a malignancy. The stellate architecture is difficult to distinguish from invasive carcinoma on mammogram, prompting biopsy of these lesions [3–7]. On histologic examination, the dense stromal fibrosis of RS can distort epithelial structures, mimicking the invasive pattern of carcinoma, especially tubular carcinoma.

The literature regarding the risk of developing subsequent *in situ* or invasive breast carcinoma following a biopsy diagnosis of radial scar has been mixed. Jacobs et al. examined 99 breast biopsies with RS in a case-control study within the Nurses' Health Study [8]. Using non-proliferative breast disease as the reference category, they found a relative risk for proliferative disease without atypia (PDWA) and RS of 3.0, and a risk for PDWA alone of 1.5 (95% CI, 1.7–5.5 and 1.1–2.1, respectively). This risk was shown to increase with the presence of atypia and RS (RR 5.8 vs. 3.8 for AH alone; 95% CI, 2.7–12.7, and 2.4–5.9, respectively). In both categories, RS acted as an independent risk factor for later breast cancer development, either *in situ* or invasive carcinoma. This risk increased proportionally with both the number and size of radial scars present. The relative risk for women with PDWA and more than one RS increased to 4.3 (95% CI, 1.7–10.8), and for women with AH and more than one RS, the relative risk increased to 8.4 (95% CI, 3.1–22.9). For size determination, the study examined lesions using a cutoff value of 4.0 mm. The relative risk for women with PDWA and RS larger than 4.0 mm increased to 3.5 (95% CI, 1.7–7.3), and for women with AH and RS larger than 4.0 mm, the relative risk increased to 8.8 (95% CI, 3.5–22.0).

Sanders et al. examined 880 breast biopsies with RS, 9.2% of the overall Nashville Breast Cohort [9]. They found an overall risk of subsequent invasive breast cancer associated with a diagnosis of RS of 1.82 (95% CI, 1.2–2.7). In contrast to Jacobs et al., the Nashville investigators did not see additive risk with the presence of RS. Specifically, for biopsies containing PDWA and RS, the risk was 2.13 (95% CI, 1.3–3.5), while the risk of PDWA alone was 1.74 (95% CI, 1.2–2.5). The relative risk of biopsies with atypia and RS was 5.39 (95% CI, 2.6–11.0) vs. 4.38 (95% CI, 2.5–7.7) for those with atypia alone. The majority of biopsies had one RS, while 6.7% of biopsies contained more than one RS. This study

failed to detect any significant difference in terms of size of radial scar and risk. The authors attribute the risk of RS to the presence of the other epithelial proliferations present, either usual type epithelial proliferative disease or AH, and do not ascribe an independent risk to RS alone.

The conflicting literature leaves uncertainty over the potential risk associated with a diagnosis of radial scar, yet the increased detection of radial scar lesions by mammographic screening makes accurate risk assessment important for clinical decision making. In this study we sought to clarify the significance of radial scar lesions using the Mayo Benign Breast Disease Cohort.

## Methods

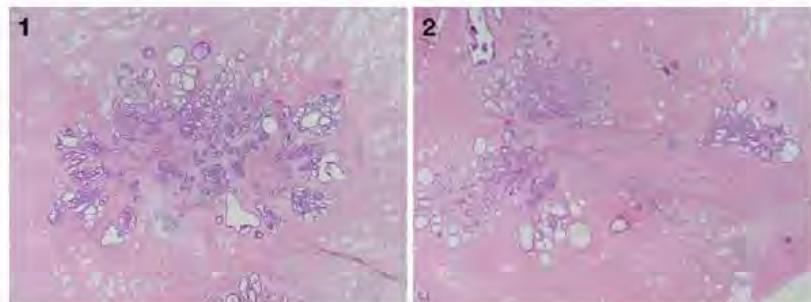
### Study population

Inclusion and exclusion criteria for the study cohort have been previously described [10]. Briefly, the study population included all women 18–85 years of age who had undergone surgical excision of a benign breast lesion at Mayo Clinic Rochester during the 25-year period from January 1, 1967 through December 31, 1991. The study cohort includes 9,376 women [11]. For 114 breast biopsy samples, the presence or absence of radial scars could not be assessed due to poor slide quality. Thus, the final cohort for this study was 9,262 women. Breast-cancer events were obtained from medical records and questionnaires. Earlier reports of the Mayo Benign Breast Disease Cohort provide detailed descriptions of the characteristics of patients and pathological specimens [10, 11].

### Histologic examination

For women who had more than one biopsy during this period, we used the earliest biopsy performed. Stored hematoxylin and eosin stained sections from each participant were evaluated by a breast pathologist (DWV) who was unaware of the initial histologic diagnoses and patient outcomes. Biopsy findings were classified according to the criteria of Page et al. into the following categories: non-proliferative fibrocytic changes, proliferative fibrocytic changes without atypia, and proliferative fibrocytic changes with atypia (atypical ductal hyperplasia, atypical lobular hyperplasia, or both) [12]. Biopsy specimens were designated as having proliferative fibrocytic changes if they contained any of the following: ductal hyperplasia (greater than mild), papilloma, radial scar, or sclerosing adenosis. Cysts, fibroadenoma, or columnar cell changes were considered non-proliferative unless they also contained one of the lesions denoted above.

**Figs. 1, 2** Typical overall stellate RS architecture; central paucicellular fibroelastic core surrounded by radiating compressed ductal structures, with peripheral accentuated cystic spaces (40 $\times$ )

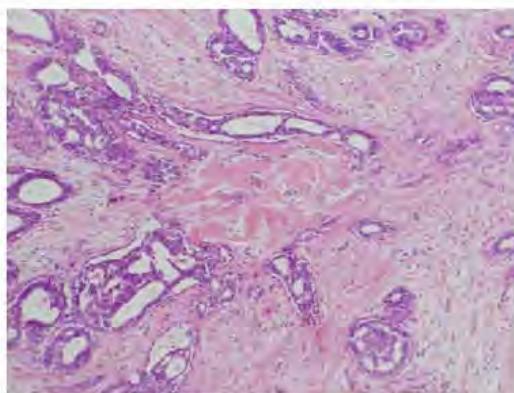


Radial scar lesions were systematically identified upon initial histopathologic review of the samples. Radial scars were defined as containing a central fibroelastic core surrounded by radiating 'arms' of compressed ducts with two-cell layers and variable epithelial hyperplasia, and classified as proliferative lesions (Figs. 1–3). Other coexisting non-proliferative (fibroadenoma, cysts) or proliferative lesions (single or multiple papillomas, sclerosing adenosis) were also counted if they appeared anywhere within the same biopsy. If AH was identified within the papilloma or in the surrounding breast parenchyma, then the case was classified as RS with AH (Fig. 4). For RS with AH, the location of the atypia (inside or outside the RS lesion) was recorded. Additional features of size and number of radial scars were also assessed. In cases of multiple RS lesions, the largest size was used for classification purposes.

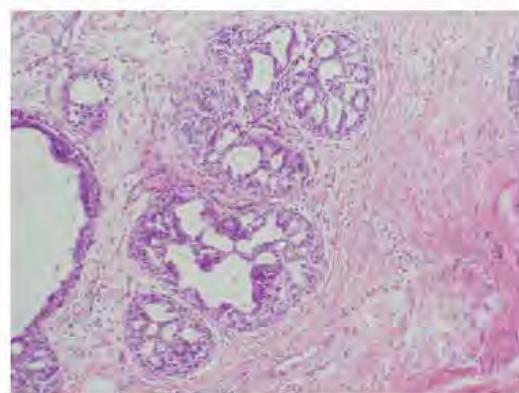
#### Statistical analysis

Data were descriptively summarized using frequencies and percents for categorical variables and means and standard

deviations for continuous variables. We compared presence of radial scars across levels of categorical variables (including age, indication for biopsy, mammogram era, extent of involution, etc.) using chi-square tests of significance. The duration of follow-up was calculated as the number of days from biopsy of the benign lesion to the date of the diagnosis of breast cancer, death, or the last contact. We estimated relative risks using standardized incidence ratios (SIRs) and corresponding 95% CI, dividing the observed numbers of incident breast cancers by population-based expected counts. We calculated these expected counts by apportioning each woman's follow-up into 5-year age and calendar period categories, thereby accounting for differences associated with these variables. We used the Iowa Surveillance, Epidemiology, and End Results (SEER) registry as the reference population because of its demographic similarities to the Mayo Clinic population (80% of cohort members reside in the upper Midwest). Over 95% of our cohort was white, equivalent to that reported in Iowa census data during the study period [13]. SIRs were calculated both overall and by subgroups defined by histology and radial scar character-



**Fig. 3** Entrapped ducts and epithelial hyperplasia near center of RS lesion (100 $\times$ )



**Fig. 4** Atypical ductal hyperplasia (ADH) arising in a RS-containing biopsy (100 $\times$ )

**Table 1** Breast cancer risk by histology and radial scars

Diagnosis	Number of eligible women (%)	Number with subsequent breast cancer diagnosis	Expected number of breast cancers	Relative risk (95% CI) <sup>a</sup>
Benign breast disease (overall)	9,262	721	521.5	1.38 (1.28–1.49)
Non-proliferative	6,244 (67.4%)	400	344.8	1.16 (1.05–1.28)
Proliferative disease without atypia (PDWA), total	2,693 (29.1%)	258	159.9	1.61 (1.42–1.82)
PDWA without RS	2,314 (86%)	215	137.0	1.57 (1.37–1.79)
PDWA with RS	379 (14.1%)	43	22.9	1.88 (1.36–2.53)
Atypical hyperplasia (AH), total	325 (3.5%)	63	16.8	3.75 (2.88–4.80)
AH without RS	265 (81.5%)	54	13.6	3.97 (2.99–5.19)
AH with RS	60 (18.5%)	9	3.2	2.81 (1.29–5.35)

<sup>a</sup> Relative risks were calculated using standardized incidence ratios, comparing the observed number of breast cancer events to those expected based on Iowa SEER rates. Expected events account for the effects of age and calendar period.

istics. We assessed potential heterogeneity in SIRs across subgroups using Poisson regression analysis, with the log-transformed expected event rate for each individual modeled as the offset term. Statistical tests were two-sided and analyses were conducted with the use of SAS (SAS) software.

## Results

### Pathologic features

Of the 9,262 women in the Mayo Benign Breast Disease Cohort, the benign lesions were classified as non-proliferative disease (NP) in 6,244 (67.4%), proliferative disease without atypia (PDWA) in 2,693 (29.1%), and atypical hyperplasia (AH) in 325 (3.5%) (Table 1). Radial scars were identified in 439 biopsies (4.7%). In these 439 biop-

sies, 382 (87.0%) contained one radial scar lesion, while 57 (13.0%) contained two or more radial scar lesions.

Of the 2,693 women with PDWA, 379 (14.1%) had at least one RS lesion; 46 (1.7%) had two or more radial scar lesions present. Of the 325 women with AH, 60 (18.5%) had at least one RS lesion; 11 of these (3.4%) had two or more radial scar lesions present.

Size of largest radial scar was available for 432 of the 439 samples. The remaining seven could not be accurately measured due to slide quality. The majority of RS, 356 (82.4%), were less than 5.0 mm in diameter, 60 (13.9%) were 5.0–9.9 mm, and 16 (3.7%) measured greater than 10.0 mm (Table 2).

RS was found concurrently with a variety of other proliferative lesions. These included sclerosing adenosis in a majority of cases (362/439, 82.5%), followed in frequency by a single papilloma (77/439, 17.5%), and multiple papillomas 3.9% (17/439). In 63 cases of RS, the

**Table 2** Breast cancer risk among women with radial scars, by age and RS features

Feature	Number	Number with subsequent breast cancer diagnosis	Expected number of breast cancers	Relative risk (95% CI) <sup>a</sup>
Overall radial scars	439	52	26.1	1.99 (1.49–2.61)
Number of radial scars				
1	382	45	22.8	1.97 (1.44–2.64)
2 or more	57	7	3.3	2.12 (0.85–4.35)
Size of largest scar <sup>b</sup>				
<5.0 mm	356	40	21.7	1.84 (1.32–2.51)
≥5.0 mm	76	9	4.0	2.26 (1.03–4.30)
Age at biopsy				
<50	156	17	8.3	2.05 (1.19–3.28)
≥50	283	35	17.8	1.97 (1.37–2.73)

<sup>a</sup> Relative risks were calculated using standardized incidence ratios, comparing the observed number of breast cancer events to those expected based on Iowa SEER rates. Expected events account for the effects of age and calendar period

<sup>b</sup> Size of largest scar could not be determined for seven of the 439 women with radial scars

**Table 3** Association of radial scars with clinical and histologic variables

Characteristic	Non-proliferative disease	Proliferative disease without RS	Proliferative disease with RS	P-value*
Overall	6,244 (67.4%)	2,579 (27.8%)	439 (4.8%)	
Age at biopsy				<0.001
<45	2,300 (76.5%)	627 (20.9%)	78 (2.6%)	
45–55	1,657 (62.7%)	832 (31.5%)	152 (5.8%)	
>55	2,287 (63.3%)	1,120 (31.0%)	209 (5.7%)	
Indication for biopsy				<0.001
Mass	3,030 (71.1%)	1,083 (25.4%)	151 (3.5%)	
Mammographic	1,410 (51.5%)	826 (34.3%)	174 (7.2%)	
Unknown	1,804 (69.7%)	670 (25.9%)	114 (4.4%)	
Time period				<0.001
Pre-mammography (1967–1981)	3,219 (72.7%)	1,064 (24.0%)	146 (3.3%)	
Post-mammography (1982–1991)	3,025 (62.6%)	1,515 (31.4%)	293 (6.0%)	
Involution				<0.001
None	1,209 (75.6%)	335 (20.9%)	56 (3.5%)	
Partial (1–74% TDLU)	2,969 (57.9%)	1,804 (35.2%)	353 (6.9%)	
Complete (75% TDLU)	1,558 (81.7%)	324 (17.0%)	26 (1.3%)	

Values presented as number (percent)

\*chi-square test of significance

additional pathology was solely non-proliferative lesions (fibroadenoma, cysts, or both).

#### Factors associated with radial scar

We examined the effect of age at biopsy on the likelihood of a RS diagnosis, dividing the cohort into those less than 45, between 45 and 55, and those over 55. As shown in Table 3, women younger than 45 were less likely to have radial scars (2.6%) than those age 45–55 (5.8%), or greater than 55 (5.7%).

Data regarding the indication for biopsy, either a mass or mammographic abnormality, were available for 71.8% of the total cohort. Of the 439 cases containing RS, 151 women (34%) had a palpable lesion, and 174 (40%) had an abnormal mammogram. For 114 (26%), the reason for the biopsy is unknown. For the 2,579 women with proliferative disease but no RS, 1,083 (42%) had a palpable lesion, and 826 (32%) had an abnormal mammogram. The remaining 670 (26%) had no known indication (proportions of palpable versus mammographic indications differ in the RS versus no RS groups—see Table 3).

Mammographic screening is thought to have increased the detection of RS. Incidence rates of RS for the pre- and post-mammographic era were examined using the year 1981 as the division point between the two eras. Women with biopsies during the post-mammography era were more likely to have radial scars than those biopsied during the pre-mammography era (6.0% vs. 3.3%) (Table 3).

Atrophy, or the degree of lobular involution, was previously found to be a risk factor in our benign breast disease (BBD) cohort [11]. This was classified in the background breast parenchyma as none (0%), partial (1–74%), or complete (≥75%) involution. Women with complete involution were less likely to have radial scars (1.3%) than those with no involution (3.5%) or partial involution (6.9%) (Table 3).

To address whether or not atypia is more frequently found in association with RS than with other forms of proliferative disease, we compared the proportion of AH with RS to that of AH with all other proliferative disease. Atypia was found at slightly higher rates in biopsies with radial scar (60/439, 13.7%), than in biopsies with other forms of proliferative breast disease without RS, such as ductal hyperplasia, papilloma or sclerosing adenosis (265/2579, 10.3%). Regarding the type of atypia arising in the setting of RS, 25 (41.7%) contained only atypical ductal hyperplasia (ADH), 31 (51.7%) with only atypical lobular hyperplasia (ALH), and 4 (6.6%) with both. Of the 60 cases with any type of atypia, the atypia was present inside the RS in 17 (28.3%), outside the RS lesion in 27 (45.0%), and present both inside and outside the RS in 16 (26.7%).

#### Breast cancer risk

The overall cohort of women with benign breast disease was at an increased risk of breast cancer development (RR = 1.38, 95% CI, 1.28–1.49) compared to the general

population. Out of the 9,262 women in this cohort evaluating radial scar, 721 developed subsequent breast cancer (Table 1). The breast cancer risk in women with radial scars associated with PDWA was 1.88 (95% CI, 1.36–2.53), compared to a relative risk of 1.57 (95% CI, 1.37–1.79) for women with PDWA without radial scar (Table 1). This difference is not statistically significant (test for heterogeneity,  $P = 0.29$ ). Similarly, for women with AH, the presence of radial scar lesions did not significantly modify breast cancer risk: RR = 2.81 for AH with RS (95% CI, 1.29–5.35) vs. 3.97 for AH without RS (95% CI, 2.99–5.19, test for heterogeneity,  $P = 0.33$ ). RS size and number information were also examined. Women with one radial scar had a relative risk of 1.97 (95% CI, 1.44–2.64) compared to 2.12 (95% CI, 0.85–4.35) for those with two or more radial scars (test for heterogeneity,  $P = 0.86$ ) (Table 2). The size of radial scar lesions also did not modify the risk for subsequent breast cancer, with RR 1.84 for lesions less than 5.0 mm (95% CI, 1.32–1.51) vs. 2.26 for lesions larger than 5.0 mm (95% CI, 1.03–4.30),  $P = 0.38$ . Age at biopsy with RS did not modify the risk for development of subsequent breast cancer, with RR 1.97 for women older than 50 years (95% CI, 1.37–2.73) vs. 2.05 for women younger than 50 years (95% CI, 1.19–3.28,  $P = 0.89$ ).

## Discussion

Radial scars are a distinctive form of benign, proliferative breast disease for which there are conflicting data regarding the subsequent risk of breast cancer. Our main finding is that RS does not confer increased risk over that of other

proliferative lesions, with or without atypia. Moreover, increasing size and number of RS were not found to influence the risk. Although we demonstrated a slightly increased association between RS and AH, the atypia arose more frequently within the background breast parenchyma, and not within the RS itself. Overall, these data argue against a direct premalignant role for RS over and above that of other forms of proliferative disease.

Our findings support the study of Sanders et al. reporting on 880 RS in women from the Nashville Breast Cohort from 1950 to 1986 (Table 4). Their average follow up was 20.4 years. Because the Nashville group did not define RS as a proliferative lesion, 73 biopsies containing non-proliferative changes also contained a RS. In the Nashville study, the risk associated with PDWA containing RS was 2.13 (95% CI, 1.3–3.5) (see Table 4). This study attributed the increased risk associated with RS to the coexistent PDWA, present in the majority (79.2%) of RS. This group detected a modest increase in risk for those women over 49 years of age [9]. In our study we saw no effect on subsequent cancer risk with increasing age at biopsy in women with RS. However, we did detect an increase in the incidence of RS with age.

In contrast to the two cohort studies from Nashville and Mayo, the study by Jacobs et al. found RS to be an independent risk factor for breast cancer (Table 4). This case-control study identified 99 RS in women from the Nurses' Health Study from 1976 to 1992 with an average follow up of 12 years. The relative risk attributed to RS was 3.0 (95% CI, 1.7–5.5) compared to 1.5 for PDWA alone (95% CI, 1.1–2.1). A similar increase was also detected for AH containing RS (RR = 5.8; 95% CI, 2.7–12.7) compared AH alone (RR = 3.8; 95% CI, 2.4–5.9). Additionally, an

**Table 4** Comparison of studies of risk associated with radial scar

Lesion	Mayo Benign Breast Disease Cohort	Nashville Breast Cohort	Nurses' Health Study
Total cohort	9,262	9,556	1,396 (cases-controls)
Number of women with RS	439	880	99
PDWA, total (n)	2,693	3,404	699
PDWA, no RS (n)	2,314	2,707	631
PDWA, no RS (RR)	1.57* (95% CI, 1.37–1.79)	1.74** (95% CI, 1.2–2.5)	1.5*** (95% CI, 1.1–2.1)
PDWA with RS (n)	379	697	68
PDWA with RS (RR)	1.88* (95% CI, 1.36–2.53)	2.13** (95% CI, 1.3–3.5)	3.0*** (95% CI, 1.7–5.5)
AH, total (n)	325	365	179
AH, no RS (n)	265	255	171
AH, no RS (RR)	3.97* (95% CI, 2.99–5.19)	4.38** (95% CI, 2.5–7.7)	3.8*** (95% CI, 2.4–5.9)
AH with RS (n)	60	110	31
AH with RS (RR)	2.81* (95% CI, 1.29–5.35)	5.39** (95% CI, 2.6–11)	5.8*** (95% CI, 2.7–12.7)

\*Reference group for Mayo Benign Breast Disease Cohort = Iowa SEER registry

\*\*Reference group for Nashville study = women in their cohort with non-proliferative disease and no radial scar

\*\*\*Reference group for Nurses Health Study = women in their cohort with non-proliferative disease

increase in RS size and number was also found to elevate the risk of subsequent cancer [8].

These differences may be explained, at least in part, by the different study designs; Jacobs et al. used a nested case-control design with four controls selected for each breast cancer diagnosis. The current Mayo study and the Nashville study are retrospective cohort studies. There were 99 biopsies with RS in the Nurses' Health Study compared to 439 (Mayo) and 880 (Nashville) RS lesions. Selection criteria for study participation may also differ between these large studies. As our data span the pre- and post-mammographic era, both types of presentations are represented. We demonstrate an increase in RS since mammographic screening began.

Earlier studies had classified RS as a benign sclerosing lesion lacking premalignant potential [14–17]. In a random examination of both benign breast tissue ( $n = 34$ ) and cancerous tissue ( $n = 34$ ), Anderson et al. attributed the presence or absence of RS more to the volume of tissue examined than to any association with carcinoma [15]. In randomized autopsy studies, Nielsen et al. found no difference between cancer-containing and noncancerous breasts in terms of presence or absence of RS [17]. A follow-up study by Nielsen et al. found an increased number of RS in the contralateral breast of women with breast carcinoma; however, no increased incidence of contralateral breast carcinoma was detected [16].

There are several reports of carcinoma arising within a RS [18–22]. These reports include *in situ* carcinomas, infiltrating ductal and lobular carcinoma [19, 21], as well as metaplastic carcinomas, including adenosquamous carcinoma [20] and spindle cell metaplastic tumors [18]. Our data do not support a unique relationship between RS and carcinoma; however, because we excluded biopsies containing carcinoma, we cannot directly comment on such isolated case report findings as these. Since most consider atypical hyperplasia to represent a direct precursor of carcinoma, one would expect an increased association between RS and AH if RS was either a precursor or promoter of neoplastic change. While our study did find a slight increased association with AH and RS-containing biopsies, the AH did not preferentially arise within RS itself. This lack of contiguity with RS and atypia would argue against RS as a direct precursor of subsequent carcinoma.

The etiology of RS lesions remains unclear. Because of their scar-like appearance, they are hypothesized to represent a healing process in breast tissue, with the central relatively acellular core representing a later phase of development [15, 23]. Molecular evidence to characterize the behavior and biologic potential of RS has found similar patterns in both RS and invasive carcinoma for mRNA expression of factors involved in formation of vascularized stroma [24]. Iqbal et al. detected subtle differences in

estrogen receptor and Ki67 expression between RS lesions containing epithelial hyperplasia and areas of epithelial hyperplasia outside of the RS lesion, with the RS containing focal clonal areas and slightly diminished estrogen receptor expression [25]. While these findings may suggest that breast tissue containing RS is somehow permissive of proliferation leading to development of carcinoma, clinical studies, including ours, have failed to demonstrate a link with carcinoma [9, 12, 15–17, 26].

In summary, with increased use of mammographic screening, RS has emerged as an important, radiographic mimic of breast cancer. Determining the accurate predictive value of RS diagnosis on breast biopsy has thus become increasingly important. Our data show that RS does not confer increased risk over that of other proliferative lesions, with or without atypia. In our large cohort study, RS emerges as an interesting histopathologic entity, with its greatest clinical significance being the degree to which it generates worrisome mammography findings.

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## Association Between Cyclooxygenase-2 Expression in Atypical Hyperplasia and Risk of Breast Cancer

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### Background

The cyclooxygenase-2 (COX-2) enzyme, which is induced by inflammatory and mitogenic stimuli, plays a protumorigenic role in several human cancers. COX-2 is overexpressed in invasive and *in situ* breast cancers. Atypical hyperplasia in breast tissue, although benign, is associated with a high risk of breast cancer. We investigated whether COX-2 overexpression in atypical hyperplasia is associated with the risk of subsequent breast cancer.

### Methods

COX-2 expression was assessed immunohistochemically in archival sections from 235 women with atypia whose biopsy specimens were obtained at the Mayo Clinic from January 1, 1967, through December 31, 1991. COX-2 expression was scored as 0 (negative), 1+ (weak), 2+ (moderate), or 3+ (strong). Risk factor information and follow-up for breast cancer events were obtained via a study questionnaire and the medical records. All statistical tests were two-sided.

### Results

Forty-one (17%) of the 235 women developed breast cancer during a median follow-up of 15 years. Moderate (category 2+) or strong (category 3+) COX-2 expression was identified in 71 (30%) and 34 (14%) of the 235 samples, respectively. The risk for developing breast cancer, relative to a control population (the Iowa Surveillance, Epidemiology, and End Results registry), increased with increasing COX-2 expression (relative risk [RR] = 2.63, 95% confidence interval [CI] = 1.56 to 4.15, for those with negative or weak COX-2 expression; RR = 3.56, 95% CI = 1.94 to 5.97, for those with moderate expression; and RR = 5.66, 95% CI = 2.59 to 10.75, for those with strong expression;  $P = .07$ ). Overexpression of COX-2 was statistically significantly associated with the type of atypia (lobular vs ductal,  $P < .001$ ), number of foci of atypia in the biopsy ( $P = .02$ ), and older age at time of biopsy ( $>45$  years,  $P = .01$ ).

### Conclusions

COX-2 appears to be a biomarker that further stratifies breast cancer risk among women with atypia and may be a relevant target for chemoprevention strategies.

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Women with atypical hyperplasia are at high risk for the development of breast cancer, with a cumulative incidence approaching 25% at 20 years after biopsy examination (1,2). Clinical practice would benefit from the identification of additional predictive features for women with atypia that will enable better risk stratification for these women and from the identification of functional biologic targets that could be modified by chemoprevention strategies.

Cyclooxygenase (COX) enzymes catalyze the synthesis of bioactive prostaglandins from arachidonic acid, which is derived from membrane phospholipids (3). Cyclooxygenase-2 (COX-2), induced in response to various inflammatory and mitogenic stimuli, has been shown to play a protumorigenic role in preclinical models of several tumor systems (4–6). Moreover, it is overexpressed in several human cancers and their precancerous lesions (7). Ristimäki et al. (8) analyzed COX-2 expression by immunohistochemistry in 1576 invasive breast cancer specimens and found moderate to strong expression in 37% of the cancers. In ductal carcinoma *in situ*, the frequency of COX-2 overexpression appears to be even higher (9,10). Many groups have shown an association between COX-2 expression and an aggressive phenotype in *in situ* and invasive breast cancer (8–15).

In animal models, COX inhibitors suppress experimental breast cancers (6,7), and several epidemiologic studies in humans have shown that nonsteroidal anti-inflammatory drug (NSAID) use is associated with a reduced incidence of breast cancer (3,16,17).

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## CONTEXT AND CAVEATS

### Prior knowledge

Cyclooxygenase-2 (COX-2) plays a protumorigenic role in several human cancers and is overexpressed in invasive and *in situ* breast cancers. Atypical hyperplasia in breast tissue, although benign, is associated with a high risk of breast cancer.

### Study design

The relationship between the risk of breast cancer and COX-2 expression in archival specimens from women with atypical hyperplasia and a 15-year follow-up was assessed.

### Contribution

The risk for developing breast cancer, relative to a control population (the Iowa Surveillance, Epidemiology, and End Results registry), increased with increasing COX-2 expression, in a borderline statistically significant manner. Overexpression of COX-2 was statistically significantly associated with the type of atypia (lobular vs ductal), with number of foci of atypia in the biopsy, and with older age at time of biopsy ( $\geq 45$  years).

### Implications

COX-2 may be a biomarker that further stratifies breast cancer risk among women with atypia and may be a relevant target for chemoprevention strategies.

### Limitations

Tissue-based biomarker studies, such as this study, are limited by semiquantitative and subjective evaluation of COX-2 status, which is further complicated by the variable nature of immunoreactivity.

Because of the relevance of COX-2 to breast cancer biology, including its presence in preinvasive lesions, we hypothesized that COX-2 expression is increased in atypia and that overexpression is a risk factor for the progression of breast cancer. Accordingly, we studied COX-2 expression in a cohort of women with atypical hyperplasia for whom we have long-term cancer follow-up information.

## Participants and Methods

### Study Population

Entry criteria for the study cohort have been described previously (1,2). Briefly, this study included all women aged 18–85 years who had a benign breast biopsy that was surgically excised at the Mayo Clinic from January 1, 1967, through December 31, 1991. The initial cohort included 9087 women (1). With additional follow-up, data for 9376 women were available for this analysis, 331 (3.5%) of whom had atypical hyperplasia (2). Archival paraffin-embedded, formalin-fixed tissue for COX-2 staining was available for 235 of the 331 women.

### Risk Factor Information and Follow-up

Follow-up for breast cancer events and risk factor information were obtained for all 235 women with atypia through Mayo medical records and a study questionnaire (1,2). Family history was collected via respondent questionnaires and medical record abstraction and classified as negative, strong, or weak. Criteria for a strong family history were at least one first-degree relative with breast

cancer diagnosed before the age of 50 years or two or more relatives with breast cancer, with at least one being a first-degree relative. Any lesser degree of family history was considered to be weak.

All protocol procedures and patient contact materials were reviewed and approved by the Institutional Review Board of the Mayo Clinic. Return and completion of the patient contact materials were considered to be implied consent.

### Histology

All archival hematoxylin- and eosin-stained sections were evaluated by the study breast pathologist (DWV), without knowledge of the original histologic diagnosis or patient outcome. A diagnosis of atypical ductal hyperplasia or atypical lobular hyperplasia was based on the criteria of Page et al. (18) and Page and Rogers (19). For each example of atypical hyperplasia, the number of separate foci was defined (2). Multifocal atypia required the identification of atypia in more than one terminal duct lobular unit, as defined by clear separation from another terminal duct lobular unit by nonspecialized interlobular mammary stroma.

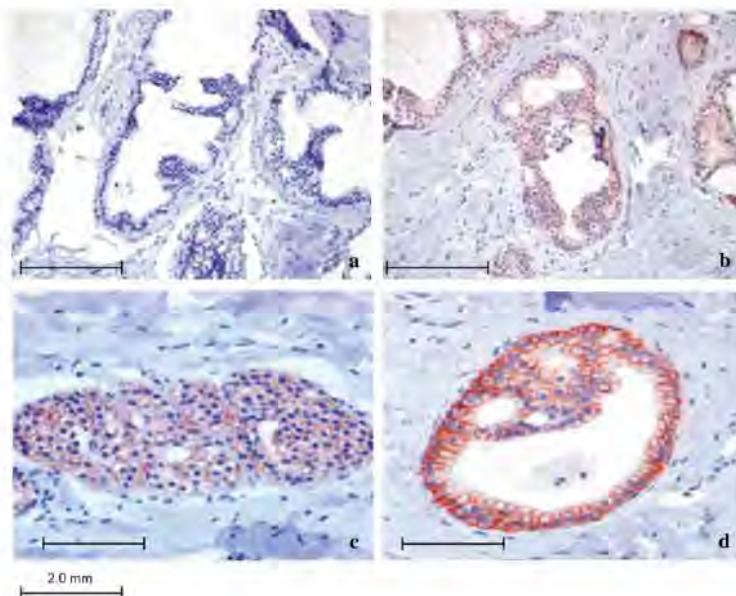
### Immunostaining

Five-micrometer sections of formalin-fixed, paraffin-embedded samples were deparaffinized with three changes of xylene, rehydrated in an ethanol series (100%, 95%, and 70% ethanol), and rinsed well in running distilled water. Slides were then placed in preheated epitope retrieval buffer (1 mM EDTA, pH 8.0) for 30 minutes, cooled in the buffer for 5 minutes, and rinsed for 5 minutes in running distilled water. An automated slide stainer (AS100 Autostainer Plus, DAKO, Carpinteria, CA) was used for all slides at room temperature as follows. Sections were first incubated in a solution of 3% H<sub>2</sub>O<sub>2</sub> in ethanol for 5 minutes to inactivate endogenous peroxides and then incubated in primary mouse anti-human COX-2, Clone CX-294, monoclonal antibody (1:100 dilution; M3617, DAKO) for 30 minutes. Sections were rinsed with TBST 10× wash buffer (S3006, tris-buffered saline with Tween 20, DAKO). Sections were then incubated with a peroxidase-labeled polymer conjugated to goat anti-mouse, anti-rabbit immunoglobulins (EnVision+ Dual Link System-HRP, K4061, DAKO) for 15 minutes. The slides were rinsed with TBST wash buffer, incubated in Nova Red (Vector Laboratories, Burlingame, CA) for 5 minutes, and counterstained with modified Schmidts' hematoxylin for 5 minutes and rinsed for 3 minutes in tap water to set the hematoxylin counterstain. Specimens were dehydrated through a graded ethanol series (70%, 95%, and 100% ethanol), cleared in three changes of xylene, and mounted with a permanent mounting medium. The positive control was colon cancer tissue; the negative control was normal colonic epithelial tissue. All samples were stained simultaneously over a 2-day period.

### Evaluation of COX-2 Immunostaining

COX-2 immunostaining was analyzed by the study breast pathologist (DWV), who had no knowledge of patient outcome. The following criteria were established before the reading of the samples (by AR and DWV) (8): 0 = no staining; 1+ = weak, barely perceptible staining in a patchy cytoplasmic pattern; 2+ = moderate intensity staining, usually cytoplasmic, with focal plasma membrane distribution; 3+ = strong immunoreactivity with distinct plasma

**Figure 1.** Cyclooxygenase-2 staining patterns in atypia. Representative breast atypia samples are shown. Other samples with corresponding scores were similar. a) Category 0 (no) staining. b) Category 1+ staining. c) Category 2+ staining. d) Category 3+ staining. Original magnification was  $\times 200$ . Scale bar = 2.0 mm.



membrane accentuation (Figure 1). Because of the small number of breast cancer events in samples with 0 staining, categories 0 and 1+ were combined for all analyses.

#### Statistical Analyses

Data were summarized descriptively by use of frequencies and percentages for categorical variables and medians and interquartile ranges (IQRs) for continuous variables. We compared distributions of demographic and clinical attributes from patients with and without available formalin-fixed tissue (for all 331 women with atypia) and across all levels of staining intensity (for the 235 women with COX-2 immunostaining results) by use of  $\chi^2$  tests.

The length of follow-up for each woman in the study was calculated as the number of days from her benign biopsy to the date of her breast cancer diagnosis, death, or last contact. We estimated relative risks (RRs) on the basis of standardized incidence ratios, by dividing the observed numbers of incident breast cancers by population-based expected values. This approach allowed us to compare rates of breast cancer in our cohort with that of the general population rather than an internal referent group, recognizing that all women in our cohort were at some increased risk of breast cancer from their diagnosis of atypical hyperplasia. Expected values were calculated by apportioning each woman's person-years of follow-up into 5-year age and calendar-period categories and multiplying these by the corresponding breast cancer incidence rates from the Iowa Surveillance, Epidemiology, and End Results registry (1). This reference population was chosen because of its demographic similarities to the Mayo Clinic population (80% of cohort members reside in the upper Midwest). Potential heterogeneity in standardized incidence ratios across levels of COX-2 staining was assessed by use of Poisson regression analysis, with the log-transformed expected event rate for each individual modeled as the offset term. We displayed observed

event rates by use of cumulative incidence curves, accounting for the effects of death as a competing risk (20). We assessed whether COX-2 staining intensity was associated in a dose-response manner with cumulative incidence by use of tests for trend, which were calculated via Cox proportional hazards regression analysis. We tested for departures from the proportional hazards assumption by use of tests of interaction with follow-up time and found no evidence of nonproportionality. Among breast cancer patients, we compared time to cancer across levels of immunostaining with analysis of variance (ANOVA) methods and laterality of breast cancer relative to the original atypia across levels of immunostaining by use of  $\chi^2$  tests. All statistical tests were two-sided, and all analyses were conducted with the SAS (SAS Institute, Inc., Cary, NC) software system.

## Results

#### COX-2 Immunostaining of Atypia Samples

Among the original cohort of 331 women with atypical hyperplasia (2), the distributions of breast cancer status, age at benign biopsy, family history of cancer, and (for breast cancer patients) time to diagnosis were not statistically significantly different between the 235 patients with formalin-fixed tissue available for COX-2 staining and the 96 patients without available tissue ( $\chi^2 P > .05$  for each attribute). Among specimens from the 235 patients with available tissue, 23 (10%) showed no COX-2 staining, 107 (46%) showed category 1+ staining, 71 (30%) showed category 2+ staining, and 34 (14%) showed category 3+ staining (Figure 1). Intensity of staining was statistically significantly associated with type of atypical hyperplasia (lobular vs ductal,  $P < .001$ ; Table 1). Of the 100 women with atypical ductal hyperplasia only, 77 (or 77%) had either no or weak (categories 0 or 1+) COX-2 staining, 13 (13%) had moderate (category 2+) staining, and 10 (10%) had strong (category 3+) staining.

**Table 1.** Association of cyclooxygenase-2 staining intensity with demographic and clinical variables, among women diagnosed with atypical hyperplasia\*

Variable	COX-2 staining category			P value†
	0-1+ (n = 130)	2+ (n = 71)	3+ (n = 34)	
Age at benign biopsy, No. (%)				.01
<45 y	24 (80.0)	6 (20.0)	0 (0.0)	
45-55 y	44 (58.7)	21 (28.0)	10 (13.3)	
>55 y	62 (47.7)	44 (33.8)	24 (18.5)	
Family history of breast cancer‡, No. (%)				.95
None	74 (54.4)	41 (30.1)	21 (15.4)	
Weak	22 (53.7)	14 (34.1)	5 (12.2)	
Strong	26 (57.8)	12 (26.7)	7 (15.6)	
No. of atypical foci, No. (%)				.02
1	84 (64.1)	31 (23.7)	16 (12.2)	
2	31 (47.7)	25 (38.5)	9 (13.8)	
≥3	15 (38.5)	15 (38.5)	9 (23.1)	
Calcifications, No. (%)				.97
Yes	40 (54.1)	23 (31.1)	11 (14.9)	
No	90 (55.9)	48 (29.8)	23 (14.3)	
Involution status§, No. (%)				.27
None	9 (64.3)	3 (21.4)	2 (14.3)	
Partial	104 (58.1)	51 (28.5)	24 (13.4)	
Complete	14 (38.9)	14 (38.9)	8 (22.2)	
Type of atypical hyperplasia, No. (%)				<.001
ALH	47 (38.5)	53 (43.4)	22 (18.0)	
ADH	77 (77.0)	13 (13.0)	10 (10.0)	
ALH and ADH	6 (46.2)	5 (38.5)	2 (15.4)	
Indication for biopsy  , No. (%)				.14
Lump	48 (50.5)	36 (37.9)	11 (11.6)	
Mammogram	78 (57.8)	35 (25.9)	22 (16.3)	
Vital status, No. (%)				.09
Deceased	38 (52.1)	19 (26.0)	16 (21.9)	
Alive	92 (56.8)	52 (32.1)	18 (11.1)	
Year of biopsy, No. (%)				.22
1967-1971	6 (66.7)	2 (22.2)	1 (11.1)	
1972-1976	12 (48.0)	12 (48.0)	1 (4.0)	
1977-1981	15 (51.7)	7 (24.1)	7 (24.1)	
1982-1986	33 (49.3)	21 (31.3)	13 (19.4)	
1987-1991	64 (61.0)	29 (27.6)	12 (11.4)	

\* COX-2 = cyclooxygenase-2; ALH = atypical lobular hyperplasia; ADH = atypical ductal hyperplasia. Values expressed as number (percent).

†  $\chi^2$  test of statistical significance.

‡ Family history was available for 222 of the 235 women. Criteria for a strong family history were at least one first-degree relative with breast cancer diagnosed before the age of 50 years or two or more relatives with breast cancer, with at least one being a first-degree relative. Any lesser degree of family history was considered to be weak.

§ Involution status was available for 229 of the 235 women.

|| Indication information was available for 230 of the 235 women.

In contrast, of the 122 women with only atypical lobular hyperplasia, 47 (39%) had no or weak staining, whereas 53 (43%) had moderate staining and 22 (18%) had strong staining ( $P < .001$ ). Strong immunostaining was also more likely with increasing patient age: no tumor from the 30 women who were younger than 45 years at time of initial biopsy had strong COX-2 staining and only six (20%) had moderate staining. In contrast, among tumors from the 130 women who were older than 55 years at biopsy, 24 (19%) had strong staining and an additional 44 (34%) had moderate COX-2 staining ( $P = .01$ ). Finally, the strength of COX-2 immunostaining was associated with increased numbers of atypia foci. Among the 39 patients with three or more foci, nine (23%) showed strong COX-2 staining and 15 (39%) showed moderate staining. In contrast, of the 131 women who had only a single focus of atypia, 16 (12%) had strong COX-2

staining and 31 (24%) had moderate staining ( $P = .02$ ). The degree of COX-2 immunoreactivity was not associated with family history of breast cancer, calendar year of biopsy, or clinical indication for biopsy (palpable lump vs mammographic abnormality).

COX-2 staining was not limited to atypical foci. Of the 235 subjects in our cohort, 216 (92%) had staining detected in benign lobules, but it was heterogeneous within tissue sections and usually weak or moderate in intensity (category 0, 1+, or 2+ in 202 [94%] of the 216 patients). A total of 179 patients also had staining in (nonatypical) proliferative lesions, such as usual-type duct hyperplasia and adenosis that were present in the tissue sections along with atypia. Most of these lesions were also weakly immunoreactive (category 0 or 1+ in 131 [73%], category 2+ in 43 [24%], and category 3+ in five [3%] of the lesions).

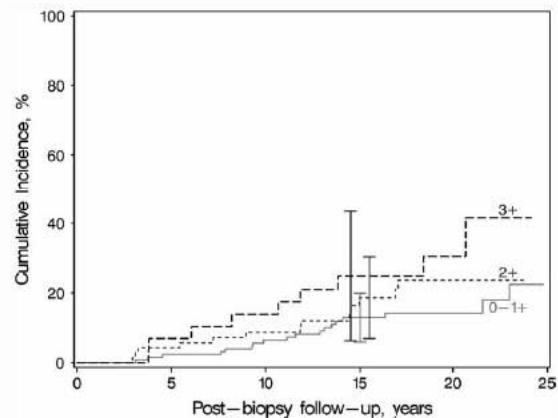
### Association of COX-2 Expression With Breast Cancer Risk

Forty-one (17%) of the 235 women with atypia in this study developed breast cancer during a median follow-up of 15 years. Figure 2 illustrates the cumulative incidence of breast cancer as a function of follow-up interval, stratified by category of COX-2 staining intensity. A positive association of borderline statistical significance was observed between COX-2 staining and the subsequent development of breast cancer (test for trend  $P = .07$ , Cox proportional hazards regression). Risks of developing breast cancer after 15 years of follow-up among women with atypia were as follows: for atypia with no or only weak COX-2 staining, 13% (95% confidence interval [CI] = 6% to 20%); with moderate staining, 19% (95% CI = 7% to 30%); and with strong staining, 25% (95% CI = 6% to 43%). After a follow-up of 20 years, risks of developing breast cancer for atypia with category 0 or 1+, category 2+, or category 3+ staining were 14% (95% CI = 7% to 22%), 24% (95% CI = 10% to 37%), and 31% (95% CI = 8% to 53%), respectively. Poisson regression analyses that compared the observed number of events with the expected number and accounted for age and calendar period also revealed a marginally statistically significant dose-response relationship overall between staining intensity and risk of breast cancer, compared with that of the control population (for category 0 or 1+ COX-2 staining, RR = 2.63, 95% CI = 1.56 to 4.15; for category 2+ staining, RR = 3.56, 95% CI = 1.94 to 5.97; and for category 3+ staining, RR = 5.66, 95% CI = 2.59 to 10.75) (Table 2, test for heterogeneity of relative risks  $P = .07$ ). Results were similar, although slightly attenuated, after further adjustment for number of atypical foci and type of atypia (atypical lobular hyperplasia or atypical ductal hyperplasia) (data not shown).

### COX-2 Staining and Cancer Features

Among the 41 women who developed breast cancer during follow-up, 32 had invasive disease, eight had in situ cancer, and one had disease of an unknown type. We compared COX-2 staining intensity (categories 0 and 1+ vs categories 2+ and 3+) in the affected women by cancer type (invasive vs in situ) and found no difference (14 of the 18 women [78%] with category 0 or 1+ staining had invasive disease, as did 18 of the 22 [82%] with category 2+ or 3+ staining,  $\chi^2 P = .75$ ).

We next examined the time to breast cancer by COX-2 staining intensity. Among the 41 women who developed breast cancer, the median time to breast cancer was 11.6 years (IQR = 7.2–14.5 years). Among the 18 women who developed breast cancer in the category 0 or 1+ COX-2 staining group, the median time to breast cancer was 11.4 years (IQR = 7.9–13.7 years); among the 23 who devel-



**Figure 2.** Cumulative breast cancer incidence among women with atypical hyperplasia, accounting for death as a competing risk. Data were stratified by level of cyclooxygenase-2 staining intensity (categories 0–1+, 2+, and 3+). Ninety-five percent confidence intervals about the cumulative incidence estimates at year 15 of follow-up are provided for reference. The number of subjects at risk by year of follow-up and staining intensity levels are as follows: baseline, 0–1+, n = 130; baseline, 2+, n = 71; baseline, 3+, n = 34; year 15, 0–1+, n = 63; year 15, 2+, n = 30; year 15, 3+, n = 11. Test for trend in risk across levels of intensity was used ( $P_{trend} = .07$ , Cox proportional hazards regression analysis).

oped cancer in the category 2+ or 3+ staining group, the median time to breast cancer was 11.8 years (IQR = 5.5–15.0 years) (ANOVA  $P = .87$ ). Side of breast cancer and side of atypia were known for 34 of the 41 women (14 in the category 0 or 1+ staining group and 20 in the category 2+ or 3+ group). In the category 0 or 1+ group, sidedness was equally distributed (seven ipsilateral and seven contralateral breast cancers). In the category 2+ or 3+ group, there were 13 (65%) ipsilateral and seven (35%) contralateral breast cancers; although seemingly different, these proportions were not statistically significantly different from each other ( $\chi^2 P = .38$ ).

### Discussion

We studied COX-2 expression in a well-characterized cohort of women with atypical hyperplasia who were followed for breast cancer events for a median of 15 years. Among the atypias from women in this cohort, 44% had moderate or strong COX-2 expression,

**Table 2.** Association of cyclooxygenase-2 staining intensity with risk of breast cancer after the diagnosis of atypical hyperplasia\*

COX-2 staining intensity	No. of women	No. of person-years	No. of observed events	No. of expected events†	RR (95% CI)‡
All women	235	3265	41	12.4	3.31 (2.38 to 4.49)
Staining category					
0–1+	130	1869	18	6.9	2.63 (1.56 to 4.15)
2+	71	1004	14	3.9	3.56 (1.94 to 5.97)
3+	34	391	9	1.6	5.66 (2.59 to 10.75)

\* COX-2 = cyclooxygenase-2; RR = relative risk; CI = confidence interval.

† Number of events expected on the basis of Iowa Surveillance, Epidemiology, and End Results data.

‡ Standardized incidence ratio and 95% confidence intervals, comparing observed number of breast cancers with those expected. All results account for age and calendar period.

and there was a borderline statistically significant increase in risk of later breast cancer associated with increasing levels of COX-2 expression ( $P = .07$ ). Atypias with more than one involved focus, which have the highest likelihood of progression to a later breast cancer (2), were more likely than those with just one involved focus to express COX-2.

There is strong biologic rationale underlying COX-2 as a relevant biomarker in breast carcinogenesis. COX-2, which is induced by mitogenic and inflammatory stimuli, has many protumorigenic downstream effects, including enhanced proliferation, enhanced angiogenesis, resistance to apoptotic cell death, immunosuppression, promotion of invasion, and metastasis (3–7,11,12). COX-2 is overexpressed in both invasive breast cancer and ductal carcinoma in situ, and overexpression is associated with aggressive histologic and clinical features (8–15). COX-2 is also overexpressed in preinvasive lesions in multiple tumor systems, including Barrett esophagus, colorectal adenomas, and cervical intraepithelial neoplasia (3,6,7).

We identified a relationship between older age at diagnosis of atypia and COX-2 overexpression. Specifically, only 20% of women who were younger than 45 years at diagnosis had atypias with moderate or strong COX-2 expression compared with 41% of women aged 45–55 years and 52.3% of women older than 55 years. Interestingly, these age-dependent differences in COX-2 induction could result in age-related changes in aromatase expression. The enzyme aromatase, encoded by the *CYP19* gene, synthesizes estradiol from androgenic precursors (7). Aromatase is present in breast tissue, and its levels are higher in or near breast cancers (21,22). It has been shown (23,24) that prostaglandin E<sub>2</sub>, which is produced by enzymes downstream of COX-2, stimulates transcription of the *CYP19* gene. Thus, increased COX-2 expression and the resultant increased mammary aromatase activity would be expected to increase local estrogen concentrations, in turn further contributing to a protumorigenic local environment (7). This mechanism may be of greater importance in postmenopausal breast cancer, where the synthesis of estrogens is dependent on aromatase in peripheral tissues, especially mammary adipose tissue (25).

We found that elevated COX-2 expression was more common in atypical lobular hyperplasia than atypical ductal hyperplasia. Perrone et al. (26) also showed recently that lobular neoplasia expressed COX-2 at high levels. Lobular neoplasia is characterized by loss of adhesion molecules such as E-cadherin, and recent work (27,28) has shown that COX-2 and prostaglandin E<sub>2</sub> induce Snail, a transcriptional repressor that silences the E-cadherin gene *CDH1*. Our data in atypia suggest that COX-2 may be responsible, at least in part, for the protumorigenic loss of adhesion molecules that occurs in lobular neoplasia of the breast.

Many studies [for review, see Harris et al. (29)] have explored whether administration of NSAIDs, which inhibit COX-2, is associated with the subsequent risk of breast cancer. Recent results from a case-control study (17) show that users of selective COX-2 inhibitors and nonselective inhibitors, including regular aspirin, ibuprofen, or naproxen, had a reduced risk of subsequently developing breast cancer, whereas users of acetaminophen did not. A previous meta-analysis (16) found a link between regular use of NSAIDs and reduction in breast cancer risk. These data supplement the extensive literature that supports the use of selective

COX-2 inhibitors as chemoprevention agents for other cancers, most notably gastrointestinal malignancies.

This study has several limitations. Tissue-based biomarker studies, such as this study, are necessarily limited by semiquantitative and subjective evaluation of COX-2 status, which is further complicated by the variable and generally weak character of immunoreactivity. More quantitative approaches, such as quantitative reverse transcriptase-polymerase chain reaction or western blotting, typically require fresh frozen tissue and cannot accurately localize the COX-2 signal in the tissue because of the small size of the atypical foci. The advantages of immunohistochemistry are that we can localize the COX-2 signal to the atypia foci and that we can use archival paraffin-embedded material from patients with adequate follow-up to determine later breast cancer events. One member of our investigative team (AR) has compared various antibodies and approaches in cancer samples in which COX-2 mRNA levels had been measured (30,31). In general, polyclonal antibodies tended to have relatively weaker staining with higher levels of non-specific staining than monoclonal antibodies (A. Ristimäki, MD, PhD, unpublished data, 2005); thus, we used a monoclonal antibody in this study. An additional challenge is that atypia lesions are small (generally <0.2 cm in diameter) and focal, thereby rendering our assay vulnerable to sampling artifacts. Hence, the clinical utility of immunohistochemical assessment of COX-2 will require additional research to establish assay consistency and reproducibility. Nevertheless, we were able to study a sizable cohort of women with atypical hyperplasia who had long follow-up time for the subsequent development of breast cancer. The association between COX-2 status and outcome, as well as the observed dose-effect relationship between development of breast cancer and the level of COX-2 staining in atypias, indicates that COX-2 may have utility as a predictive biomarker. Findings in this study support the association between NSAID treatment and risk reduction in breast cancer and also the potential to devise individualized chemoprevention approaches based on patient-specific biomarker assays. For example, in a recent study, Chan et al. (32) showed that efficacy of aspirin as a chemoprevention strategy for colorectal carcinoma was limited to patients with tumors that express COX-2.

In summary, we found moderate to strong COX-2 expression in 44% of atypical hyperplasia samples from a well-characterized patient cohort. Samples with three or more foci of atypia, which are associated with increased risk of subsequent breast cancer (2), had stronger COX-2 staining. However, the relationship between COX-2 staining intensity and the risk of subsequent breast cancer was of only borderline statistical significance ( $P = .07$ ).

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**Lobular involution: localized phenomenon or field effect?**

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As women age, the lobules in their breasts undergo involution. We have shown that, in women with benign breast disease, progressive involution assessed near the benign lesion is associated with lower breast cancer risk. However, it is unknown whether the extent of involution is variable or uniform across the entire breast. We compared involution across the four quadrants of both breasts for fifteen women undergoing bilateral prophylactic mastectomy. One pathologist classified involution extent as none (0% involved lobules), mild (1-24%), moderate (25-74%), or complete ( $>/=75\%$ ). We assessed intra-woman concordance using intraclass correlation coefficients (ICCs), kappa coefficients, and pairwise comparisons of agreement. We found strong intra-woman concordance of involution across the eight quadrants of breast tissue (ICC = 0.75, 95% CI 0.59, 0.89). Our study suggests that lobular involution is a homogeneous process, supporting the use of involution measures from a single benign biopsy as a component in breast cancer risk assessment paradigms.

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## Lobular Involution: Localized Phenomenon or Field Effect?

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Key Words: breast cancer, lobular involution

### ABSTRACT

As women age, the lobules in their breasts undergo involution. We have shown that, in women with benign breast disease, progressive involution assessed near the benign lesion is associated with lower breast cancer risk. However, it is unknown whether the extent of involution is variable or uniform across the entire breast. We compared involution across the four quadrants of both breasts for fifteen women undergoing bilateral prophylactic mastectomy. One pathologist classified involution extent as none (0% involved lobules), mild (1-24%), moderate (25-74%), or complete ( $\geq 75\%$ ). We assessed intra-woman concordance using intraclass correlation coefficients (ICCs), kappa coefficients, and pairwise comparisons of agreement. We found strong intra-woman concordance of involution across the eight quadrants of breast tissue (ICC=0.75, 95% CI 0.59, 0.89). Our study suggests that lobular involution is a homogeneous process, supporting the use of involution measures from a single benign biopsy as a component in breast cancer risk assessment paradigms.

## INTRODUCTION

The epithelium of the human breast is organized into approximately 15-20 major lobes, each comprised of terminal duct lobular units (TDLUs, or lobules) which contain the milk-producing acini. These lobules are the anatomic substructure that gives rise to breast cancer.[1] As a woman ages, her lobules involute, with a resulting reduction in the number and size of acini per lobule (Figure 1)[2-6]. In a recent study, we showed that progressive degrees of lobular involution were associated with lower breast cancer risk in women with pathologically confirmed benign lesions of the breast.[7] That study used a single assessment of involution for each woman based on the normal background lobules at the site of the biopsy. It has been suggested that extent of involution can help predict risk of breast cancer.[7,8] To do so, the extent of involution in a small tissue sample would need to be representative of the entire field of a woman's breast tissue. To our knowledge no study has examined the uniformity of involution. Thus, we sought to determine if extent of lobular involution was similar across multiple areas of a woman's breasts by studying tissue from women undergoing bilateral prophylactic mastectomy.

## METHODS

After Institutional Review Board approval, mastectomy specimens from 15 women with no personal history of cancer who had undergone bilateral prophylactic mastectomy at Mayo Clinic between 1998 and 2006 were retrieved from the Tissue Registry. A single section of fibrous breast tissue was sampled from each quadrant of both breasts, for a total of eight samples per individual. For each section, a formalin-fixed, paraffin embedded hematoxylin and eosin slide was prepared for tissue examination. The slides were labeled in a blinded manner, randomly intermixed, and provided to our breast pathologist (CR). Each specimen was categorized by the extent of lobular involution as none (0% involved lobules), mild (1-24%), moderate (25-74%), or complete ( $\geq 75\%$  involved lobules).

We calculated within-woman concordance of involution extent using intraclass correlation coefficients (ICCs).[9-10] We first binned all eight values from a woman into a single class, modeling each woman as an experimental unit. Secondary analyses considered the four readings within a breast to be potentially correlated, but assumed readings across breasts were independent. For these analyses, each woman contributed two classes of four measures, effectively modeling each breast as the experimental unit. Initial ICCs pooled the intermediate involution categories of mild and moderate into one "partial involution" category, identical to the measurement in our previous study.[7] Correlations were then re-examined using the four-level categorization of none, mild, moderate and complete.

We also calculated kappa coefficients as a measure of agreement. We used the generalized multiple-rater kappa coefficient to account for the fact that we have more than two measures within each unit,[11] resulting in a conservative, unweighted kappa that does not allow for "partial credit" due to close, but not exact, matches.

---

To aid in interpretation, we examined and summarized all pairwise comparisons of intra-woman involution values using the three level involution variable defined above (none; partial, pooling mild and moderate; complete). Each woman's eight measures resulted in 28 pairwise comparisons, or 420 total in our group of 15. We classified each paired comparison as a perfect match (the paired observations agree), partial match (the observations differ by one category), or non-match (the observations differ by two categories). Secondary analyses summarized pairwise measurements only for the four values within a breast, resulting in six breast-specific pairwise comparisons, and thus twelve for each woman and 180 overall.

## RESULTS

The mean age at mastectomy for our fifteen women was 53.9 years (range 37-72). Of the 120 assessments of involution, 9 were classified as no involution, 27 as mild, 25 as moderate, and 59 as complete.

Within-woman pairwise comparisons of involution, based on the three level involution variable, are presented in Table 1. Of the 420 total comparisons (28 for each of the 15 women), 341 (81%) were classified as perfect matches, 76 (18%) as partial matches, and 3 (1%) as non-matches. The proportion of perfect and partial matches was similar when confining the pair-wise comparisons to readings within the same breast.

We observed strong correlation among involution measures (Table 1). ICCs for both the four level and three level involution variables were identical (ICC=0.75; 95% CI=0.59,0.89). Correlations were similar when modeling each breast as the experimental unit, indicating that patterns of involution were similar within each breast and across both breasts within a woman.

Kappa statistics also demonstrated strong agreement. Using generally accepted categorizations,[12] the kappa for the three level involution variable fell into the "substantial" agreement category (kappa=0.67, 95% CI 0.59,0.75, Table 1), while the four level involution kappa fell into the "moderate" category (kappa=0.56, 95% CI 0.49,0.62).

## DISCUSSION

We found high concordance of lobular involution values across multiple areas of breast tissue in women undergoing bilateral prophylactic mastectomy. Measures of agreement incorporating the eight quadrants across both breasts were similar to those obtained when treating each breast independently, indicating uniformity of effect in the entire field of breast tissue.

We previously reported a strong dose-response association of lobular involution with lower breast cancer risk among women with benign breast disease based on the background tissue surrounding the benign lesion,[7] suggesting that assessing involution extent could improve risk prediction capabilities.[7,8] Our current findings provide evidence that the assessment of involution extent from a single area of the breast is representative of the entire field of breast tissue.

Our study is limited by the large group of specimens falling under the intermediate categories of mild and moderate involution. It is possible that concordance would increase with more objective determinations of involution. We are currently exploring quantitative measures of lobule regression that may better define the involution status of women.[13]

In summary, we observed moderate to high uniformity among measures of age-related lobular involution from multiple areas of breast tissue within a woman. This finding suggests that involution is a consistent physiological process across the field of breast tissue, supporting the use of measures from a single benign biopsy for breast cancer risk prediction.

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Table 1. Measures of concordance of involution in 15 women undergoing bilateral prophylactic mastectomy. Eight quadrants assessed per woman (four per breast).

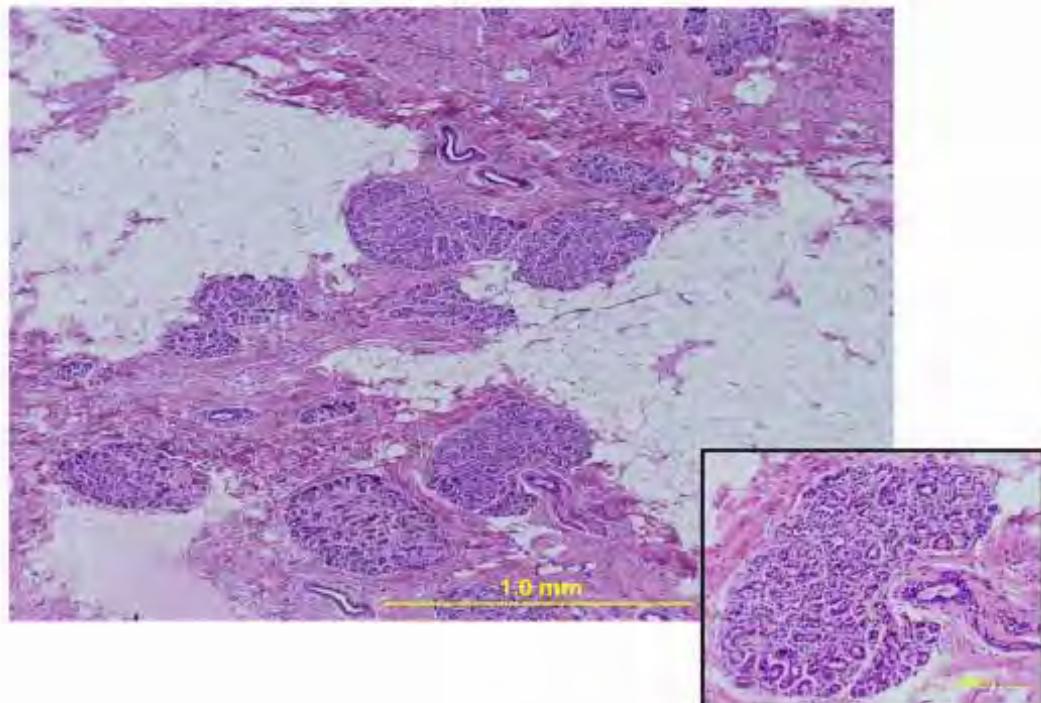
Measure	Result
<u>Treating each woman as the experimental unit</u>	
Intraclass Correlation (95% CI)	0.75 (0.59,0.89)
Kappa Coefficient (95% CI)	0.67 (0.59,0.75)
Pairwise Comparisons, N (%) <sup>1</sup>	
Perfect Matches	341 (81)
Partial Matches	76 (18)
Non-Matches	3 (1)
<u>Treating each breast as the experimental unit</u>	
Intraclass Correlation (95% CI)	0.74 (0.60,0.85)
Kappa Coefficient (95% CI)	0.66 (0.53,0.78)
Pairwise Comparisons, N (%) <sup>1</sup>	
Perfect Matches	145 (81)
Partial Matches	34 (19)
Non-Matches	1 (1)

Results are based on the three-level assessment of involution: none (0% involved lobules), partial (1-74%), and complete ( $\geq 75\%$ ). Analyses using a four-level assessment, stratifying the partial involution category into mild (1-24% involved lobules) and moderate (25-74%), yielded similar results.

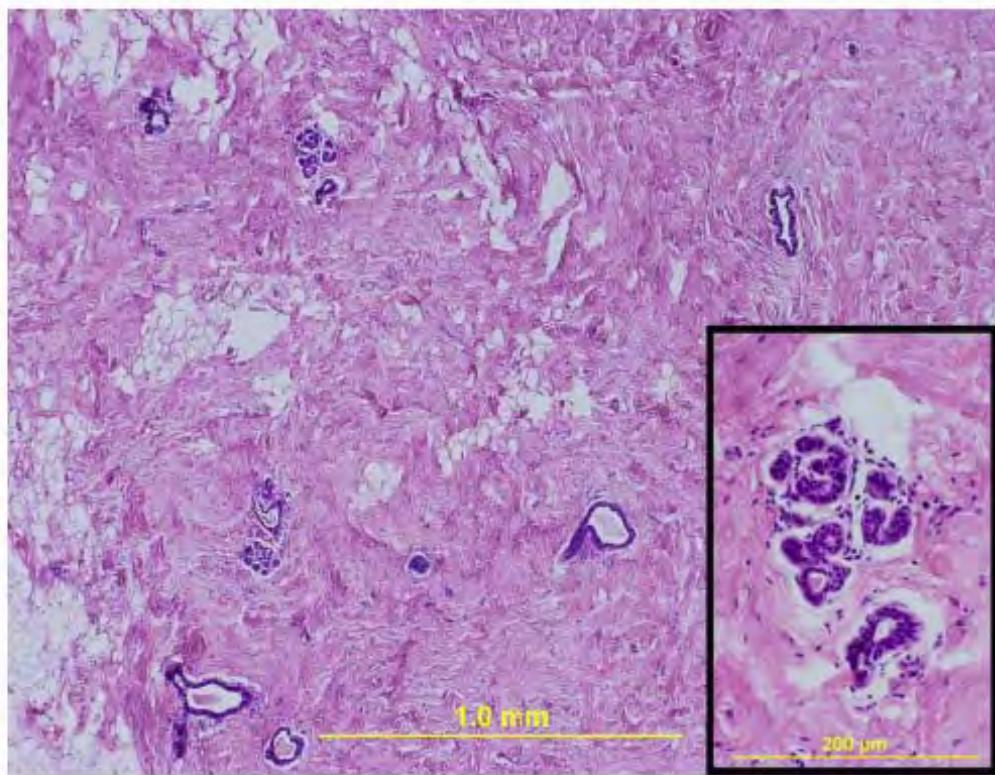
1. Pair-Wise comparisons of involution measures within a woman. When treating woman as the experimental unit, the eight involution reads result in 28 pair-wise comparisons per woman, or 420 overall in our group of 15 women. When treating each breast as an experimental unit, the four involution reads result in 6 pair-wise comparisons per breast, or 12 per woman and thus 180 overall.

Figure 1. Histologic features of age-related involution. (A) An example of breast tissue with no lobular involution, with multiple intact terminal duct lobular units, each comprised of multiple acini and specialized stroma (Inset). (B) An example of complete lobular involution with residual terminal duct lobular units, largely depleted of acini (Inset).

(a)



(b)



## Assessment of the Accuracy of the Gail Model in Women With Atypical Hyperplasia

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### ABSTRACT

#### Purpose

An accurate estimate of a woman's breast cancer risk is essential for optimal patient counseling and management. Women with biopsy-confirmed atypical hyperplasia of the breast (atypia) are at high risk for breast cancer. The Gail model is widely used in these women, but has not been validated in them.

#### Patients and Methods

Women with atypia were identified from the Mayo Benign Breast Disease (BBD) cohort (1987 to 1991). Their risk factors for breast cancer were obtained, and the Gail model was used to predict 5-year- and follow-up-specific risks for each woman. The predicted and observed numbers of breast cancers were compared, and the concordance between individual risk levels and outcomes was computed.

#### Results

Of the 9,376 women in the BBD cohort, 331 women had atypia (3.5%). At a mean follow-up of 13.7 years, 58 of 331 (17.5%) patients had developed invasive breast cancer, 1.86 times more than the 34.9 predicted by the Gail model (95% CI, 1.29 to 2.15;  $P < .001$ ). For individual women, the concordance between predicted and observed outcomes was low, with a concordance statistic of 0.50 (95% CI, 0.44 to 0.56).

#### Conclusion

The Gail model significantly underestimates the risk of breast cancer in women with atypia. Its ability to discriminate women with atypia into those who did and did not develop breast cancer is limited. Health care professionals should be cautious when using the Gail model to counsel individual patients with atypia.

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### INTRODUCTION

An accurate estimate of a woman's risk of developing breast cancer is an integral component of patient counseling. It enables physicians to tailor clinical management to the patient's needs and guide patients in the selection of appropriate medical and surgical management. Women with biopsy-confirmed atypical hyperplasia of the breast (atypia) are known to be at high risk for the development of breast cancer.<sup>1-3</sup> Widespread public awareness of breast disease along with routine use of screening mammograms has led to the increased detection of atypia on breast biopsy.<sup>1</sup> Women with atypia are often counseled to pursue heightened screening and risk reduction strategies such as chemoprevention with tamoxifen or raloxifene. To assist a woman with atypia in making an informed decision, an accurate assessment of her risk is needed.

The original Gail model was developed using data from women who were actively participating in the Breast Cancer Detection and Demonstration Project, a breast cancer screening program.<sup>4</sup> It was updated and validated across a population of women in the National Surgical Adjuvant Breast and Bowel Project P-1 study.<sup>5</sup> This updated version of the model (called model 2 in Costantino et al<sup>7</sup>) has been implemented in a variety of formats. It is incorporated in the Breast Cancer Risk Assessment Tool (BCRAT, also referred to in this article as the Gail model), which is available on the National Cancer Institute (NCI) Web site (<http://cancer.gov/bcrisktool>) and is viewed 20,000 to 30,000 times per month, suggesting strong demand for this information.<sup>8</sup> The Gail model provides individualized risk estimates of the probability that a woman with specific characteristics will develop invasive breast cancer during the next 5 years, and by age 90 years.

The Gail model incorporates information on risk factors such as age, age at menarche, age at first live birth, number of first-degree relatives with breast cancer, number of prior breast biopsies, and presence of atypia on biopsy. It is currently the main tool used for breast cancer risk assessment in patients with atypia. Despite its widespread use, the Gail model has not been validated in patients with atypia. Therefore, we evaluated the Gail model in a well-annotated, well-characterized cohort of women with atypia on open breast biopsy.<sup>1,2</sup>

## PATIENTS AND METHODS

Details of the study cohort have been previously described.<sup>1,2,8</sup> Briefly, the Mayo Benign Breast Disease (BBD) cohort comprises 9,376 women age 18 to 85 years who underwent open breast biopsy at the Mayo Clinic (Rochester, MN) between 1967 and 1991, with benign pathologic findings. Women with a history of ductal carcinoma in situ, lobular carcinoma in situ, or invasive breast cancer were excluded. Benign breast tissue from all women in the cohort was reviewed by our study pathologists (C.R. and Daniel W. Visscher, MD) without knowledge of the original histologic diagnosis or patient outcome. A diagnosis of atypia (atypical ductal hyperplasia, atypical lobular hyperplasia, or both) was made in 331 women (3.5%) using the standard criteria and histologic classification of Dupont and Page.<sup>1,3,4,6</sup> The study was approved by the institutional review board of the Mayo Clinic and all patient contact materials were reviewed and approved.

Each individual's risk factors for the development of invasive breast cancer were obtained via a study-specific questionnaire and from medical record review. The most current data available for each risk factor were used. Follow-up was calculated as the number of days from benign biopsy to the date of invasive breast cancer diagnosis, prophylactic mastectomy, death, or last contact. Last contact was either last return visit to the Mayo Clinic or the date of return of the study questionnaire, whichever occurred later. Patients were classified as having either developed invasive breast cancer or not. No women developed lobular carcinoma in situ, and those who developed ductal carcinoma in situ were included in the second of these two groups, given that the Gail model only predicts invasive breast cancer.

Using age at biopsy as the age at risk assessment, the Gail model was used to predict 5-year risk for each individual with atypia using her risk factor profile.<sup>7</sup> Risk estimates corresponding with length of follow-up for each woman (termed follow-up-specific risk estimates) were also calculated. To obtain these breast cancer risk estimates, we employed a Fortran program that was provided to us by NCI (M. Gail, J. Benichou, D. Pee, personal communication, February 2007), which contains the code comprising the underlying calculation machinery used in NCI's BC RAT. The standards used in the online Gail model were used for variables with missing data. Women with unknown age at menarche were assigned menarche at 14 years of age or older. Women with unknown age at first live birth were classified as giving birth before age 20 years. Women with missing family history were classified as having no family history. To verify agreement between the code we used and the online tool, we randomly selected 10 patients and compared the 5-year and lifetime risk estimates obtained from the code given to us to those from the online risk assessment tool. All of the estimates were in complete agreement.

The Gail model risk factors of the women with atypia were summarized using counts and percentages, or means and standard deviations, both overall and also according to invasive breast cancer status. The cumulative incidence of breast cancer was estimated using methods that corrected for the competing risk of death.<sup>11</sup> Cox proportional hazards regression models were used to assess associations between the risk of breast cancer and each of the Gail model risk factors. Hazard ratios, their 95% CIs, and *P* values assessing the associations were obtained.

Gail model predictions were summarized across the study group. Ranges of the predictions were extracted, together with means and standard deviations, for 5-year and follow-up-specific risk estimates. The distributions of the follow-up-specific risk estimates, by invasive breast cancer status, were obtained by computing the proportion of individuals whose risk predictions fell

into specified categories. A graph was obtained by plotting these percentages against the center of the risk prediction categories, and linearly interpolating the points. The 5-year risk predictions and the follow-up-specific probabilities were aggregated to obtain estimates of the number of breast cancers predicted by the Gail model, both overall and by the categories of the Gail model risk factors. The expected numbers of breast cancers were compared with the observed diagnoses by computing the ratio of observed to expected invasive breast cancers. Tests of significance and 95% CIs were obtained using the Poisson distribution.

The precision with which the Gail model predictions agreed with ultimate breast cancer events was assessed with the concordance statistic (termed *c*-statistic). This statistic is equivalent to the area under the receiver operating characteristic curve for diagnostic tests, and reflects the ability of the risk predictions to correctly order individuals relative to the timing of the observed breast cancer outcomes. For time-to-event outcomes, it is computed by forming all possible pairs of patients where the patient with the shorter follow-up time experienced breast cancer, and by tallying the number of pairs where the patient with the early event had a higher breast cancer risk score.<sup>12</sup> CIs for the *c*-statistics were obtained via a bootstrap approach. Given that several risk factors had missing data, a second set of analyses was performed to assess the accuracy and precision of the Gail model in the women with complete covariate information.

## RESULTS

Of the 9,376 patients in the Mayo BBD cohort, 331 women (3.5%) had atypia. At a mean follow-up of 13.7 years, 58 of the 331 women with atypia (17.5%) developed invasive breast cancer (the cases), eight in the first 5 years after biopsy. Among the 331 patients with atypia, 75 women (22.7%) died while in active follow-up. Nine of the deaths were among the patients who developed cancer and 66 were among the remainder of the atypia cohort.

In Table 1 we list the Gail model features for the 331 women with atypia. In Figure 1 we show the age-specific cumulative incidence of breast cancer, along with population expectations and Gail model predictions for this cohort. Table 1 also lists associations between Gail model risk factors and invasive breast cancer onset. When comparisons were made in this cohort of women with atypia, in whom 58 invasive breast cancers were observed, no statistically significant associations were apparent.

### Aggregate Performance

The Gail model predicted an average 5-year breast cancer risk of 4.2% (standard deviation, 2.7%; range, 0.3% to 18.8%). This equated to a predicted total of 13.9 breast cancers within 5 years. In this time interval, eight invasive breast cancers were observed. The ratio of observed to predicted events was 0.58 (95% CI, 0.29 to 1.15; *P* = .120).

When used to predict the risk of breast cancer by the end of the current follow-up, the mean Gail model risk was 10.5% (standard deviation, 8.2%; range, 0.4% to 54.1%). These risk estimates predicted that 34.9 women would experience an invasive breast cancer during the time for which follow-up was available. The observed count of 58 events during the observation period was significantly higher than predicted (ratio, 1.66; 95% CI, 1.29 to 2.15; *P* < .001).

Table 2 summarizes the number of events observed in our cohort and the number of events predicted by the Gail model for each Gail model risk factor. The Gail model underestimated the number of breast cancers, both overall and in the majority of the risk-factor-defined subgroups.

Table 1. Characteristics of the Women With Atypia According to Whether They Developed Breast Cancer

Characteristic	All Patients (n = 331)		No Invasive Cancer (n = 273)		Invasive Cancer (n = 58)		Hazard Ratio*	95% CI†	P‡
	No.	%	No.	%	No.	%			
<b>Age at biopsy, years</b>									
< 45	46	13.9	37	13.8	9	15.5	1.04	0.71 to 1.51	
46-55	100	30.2	78	28.6	22	37.9	1.07	0.80 to 1.42	
> 55	185	56.9	158	57.9	27	46.8	Ref		
Mean		58.0		58.7		64.5	0.99	0.96 to 1.01	.252
Standard deviation		12.0		12.5		9.0			
<b>Age at menarche, years</b>									
< 12	39	11.8	29	10.6	10	17.2	1.86	0.86 to 3.99	
12-13	119	36.0	100	36.6	19	32.8	Ref		
> 13	57	17.2	46	16.8	11	19.0	1.31	0.63 to 2.76	
Unknown	116	35.0	98	35.9	18	31.0	1.53	0.80 to 2.92	
<b>Age at first live birth, years</b>									
Nulliparous	43	13.0	35	12.8	8	19.8	1.46	0.62 to 3.38	
< 20	39	11.8	35	12.8	4	6.9	0.60	0.20 to 1.80	
20-24	104	23.3	88	32.2	16	27.6	Ref		
25-29	73	22.1	56	20.1	18	31.0	1.68	0.86 to 3.30	
≥ 30	31	9.4	27	9.9	4	6.9	0.92	0.31 to 2.75	
Unknown	41	12.4	33	12.1	8	13.8	1.78	0.75 to 4.12	
<b>First-degree relatives with breast cancer</b>									
0	200	60.4	159	58.2	41	70.7	Ref		
1	73	22.1	63	23.1	10	17.2	0.89	0.25 to 1.92	
≥ 2	14	4.2	13	4.8	1	1.7	1.18	0.76 to 1.79	
Unknown	44	13.3	38	13.9	6	10.3	1.19	0.97 to 1.45	
<b>No. of biopsies‡</b>									
1	229	69.2	189	69.2	40	69.0	Ref		
≥ 2	102	30.8	84	30.8	18	31.0	1.10	0.83 to 1.46	

Abbreviation: Ref, reference.

\*Hazard ratios and corresponding 95% CIs from a Cox proportional hazards regression model.

†P value testing the null hypothesis that all categories for the feature of interest have equal breast cancer risk.

‡As an enrollment criterion, each of the women in the cohort had at least one biopsy.

### Individual-Specific Performance

Figure 2 shows the distributions of the Gail model risk estimates for women who did and did not develop invasive breast cancer. These distributions are shown adjusted to the mean follow-up time of 13.7 years and to the mean age at atypia of 58.0 years. This was done to eliminate bias induced by the controls' having longer follow-up than the patients, given that follow-up for patients stops at the time of diagnosis of breast cancer, and to account for differential risk estimates by age at diagnosis of atypia. With a model that perfectly discriminates between groups, the two distributions would not overlap. Here, there is extensive overlap between risk estimates for the patients and non-cases. The age and follow-up-adjusted average risk predictions are slightly lower in the patients ( $10.0\% \pm 5.4\%$  v  $10.7\% \pm 7.3\%$ ), although not significantly ( $P = .465$ ).

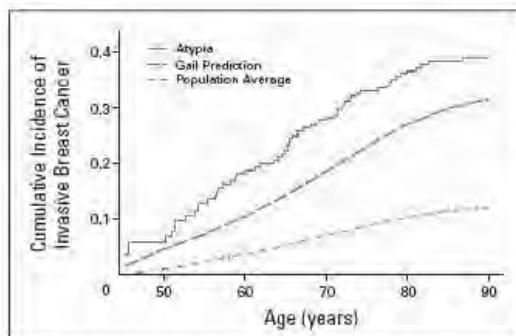
The concordance between observed and predicted invasive breast cancer events after 5 years, as measured by the c-statistic, was 0.47 (95% CI, 0.21 to 0.73), not significantly different from the value of 0.5 that would be expected by chance ( $P = .792$ ). When using the risk estimates specific to the length of follow-up, the c-statistic was 0.50 (95% CI, 0.44 to 0.55), not significantly different from the value of 0.5 expected by chance ( $P = .915$ ).

To determine the degree to which missing data affected estimates of the accuracy of the Gail model predictions, we recomputed the

prediction accuracy within the 192 individuals (58%) with complete data. As an additional sensitivity analysis, we also recomputed the prediction accuracy in all participants after imputing the value that would lead to the highest risk prediction. The observed-to-expected ratio of invasive breast cancers in those with complete data, at 1.44 (95% CI, 1.04 to 2.00), was somewhat lower than the value of 1.66 observed in all 331 women. However, this still reflected a significant discrepancy between the Gail model predictions and the observed invasive cancers among this subgroup of women with complete data ( $P = .028$ ). The c-statistic in the complete-data subset was somewhat higher (0.53) than what we observed in the entire cohort (0.50). Even when imputing in a way that leads to the highest possible number of expected cancers, the observed-to-expected ratio was still significantly inflated (1.32; 95% CI, 1.02 to 1.70;  $P = .036$ ), whereas the c-statistic (0.52) was similar to what was observed in the complete-data subset.

### DISCUSSION

We studied the Gail model in a well-defined cohort of women with atypia with an average follow-up of nearly 14 years. Measuring the performance of the model, the model slightly overpredicted the number of invasive breast cancers during the first 5 years, but



**Fig 1.** Cumulative incidence of invasive breast cancer among women with atypical hyperplasia (atypia) as a function of age. The red line represents the cumulative incidence, corrected for the competing risk of death, in the atypia cohort. For comparison, two lines representing the Gail-predicted and the baseline population risks are included. The blue line reflects the cumulative incidence predicted by the Gail model in this cohort, and the gray line represents the cumulative incidence that serves as the baseline risk for white women in the Gail model calculations.

substantially underpredicted the number of invasive breast cancers during the 13.7 years of follow-up. The individual-specific agreement between the Gail model predictions and actual breast cancer outcomes was low. For the first 5 years after biopsy the *c*-statistic was 0.47 (95% CI, 0.21 to 0.73), no better than chance alone.

During the entire 13.7 years of follow-up, the Gail model predictions were concordant with invasive breast cancer outcomes 50% of the time (95% CI, 44% to 55%), also not significantly better than chance. This finding is lower than assessments of the Gail model in other cohorts, where *c*-statistics of 0.58 to 0.59<sup>13, 14</sup> have been reported, although the upper limit of the CI approaches these previously reported values.

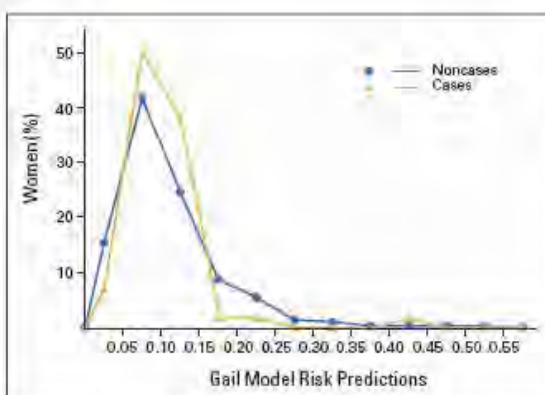
This cohort consists of a large collection of women with atypia. However, as we assessed the quality of the risk predictions of the Gail model, there were women for whom complete covariate information was not available (Table 1). When we recomputed the risk estimates in the subset of women with complete data, the estimates were similar. The *c*-statistic in the complete-data subset indicated performance similar to what was observed in the entire cohort. Even in the situation where the missing data were imputed in such a way as to produce the maximum number of predicted breast cancers, the Gail model still predicted a significantly lower number of breast cancer events than were observed. Thus, it seems unlikely that the level of missing data can explain the underestimate of breast cancer risk that is reported here.

Clinical management of women diagnosed with atypia includes quantitative breast cancer risk assessment, comprehensive discussion of risk reduction strategies, and recommendations for future breast cancer screening. The currently available risk reduction options include chemoprevention with agents such as tamoxifen or raloxifene,<sup>15, 16</sup> surgical therapy with prophylactic mastectomy, and/or lifestyle modification. Unfortunately, lifestyle modifications,

**Table 2** Comparison of Observed and Predicted Breast Cancer Events by Gail Model Risk Factors for Invasive Breast Cancer After Diagnosis of Atypia

Characteristic	Subjects		Person-Years	Observed Events	Predicted Events*	Ratio Observed:Predicted Events	95% CI
	No.	%					
Overall	331		4,543.2	58	34.9	1.66	1.29 to 2.15
Age, years							
< 46	46	13.9	678.2	9	3.9	2.28	1.19 to 4.38
46-55	160	30.2	1,540.0	22	10.7	1.90	1.36 to 3.14
> 55	185	55.9	2,325.0	27	20.3	1.15	0.91 to 1.94
Age at menarche, years							
< 12	39	11.8	582.9	10	5.0	1.99	1.07 to 3.69
12 to 13	119	36.0	1,878.7	19	15.3	1.24	0.79 to 1.95
> 13	57	17.2	841.8	11	6.8	1.62	0.90 to 2.93
Unknown	116	35.0	1,259.9	18	7.8	2.32	1.46 to 3.68
Age at first live birth, years							
Nulliparous	43	13.0	531.3	8	4.3	1.88	0.94 to 3.76
< 20	39	11.8	610.4	4	4.2	0.96	0.36 to 2.56
20-24	104	23.3	1,475.2	16	11.5	1.39	0.85 to 2.27
25-29	73	22.1	1,080.2	18	8.5	2.12	1.34 to 3.36
≥ 30	31	9.4	433.0	4	3.9	1.04	0.39 to 2.77
Unknown	41	12.4	463.2	8	2.6	3.07	1.54 to 6.15
First-degree relatives with breast cancer							
0	200	60.4	2,736.2	41	16.2	2.52	1.86 to 3.43
1	73	22.1	1,042.7	10	11.5	0.87	0.47 to 1.62
≥ 2	14	4.2	208.9	1	4.3	0.23	0.03 to 1.64
Unknown	44	13.3	556.4	8	2.8	2.15	0.97 to 4.79
Number of biopsies							
1	229	69.2	2,991.3	40	21.0	1.90	1.40 to 2.60
≥ 2	102	30.8	1,552.0	18	13.9	1.30	0.82 to 2.06

\*Calculated by applying observed person-years of follow-up to Gail model risk prediction estimates.



**Fig 2.** Distributions of Gail model risk probabilities in women with atypia who developed breast cancer (cases) and those who did not (noncases). The plot contains estimates for individualized risk at the end of the available follow-up. Given that risk predictions depend on age at benign breast disease (BBD) and length of follow-up, the risk predictions were corrected for these factors before comparison. The graph represents the percent of women whose Gail model risk predictions fell within categories ranging from 0.0 to 0.5 in 0.05 increments. Points connected by lines to facilitate comparison between case and noncase interval percentages. Though the cases received more predictions in the 10% to 15% interval than the noncases, their average risk prediction was slightly, although not significantly, lower.

such as adoption of a healthy diet, maintenance of a healthy weight, and avoidance of smoking and smoking environments do not seem to provide a substantive reduction in risk of breast cancer. Chemoprevention requires consideration of the risks and benefits of the medications and surgical intervention can be associated with significant morbidity. Gail et al<sup>17</sup> provide a useful overview regarding the risks and benefits of using tamoxifen, and demonstrate that the use of tamoxifen in women with atypia provides a net benefit. With more reliable assessments of breast cancer risk, it will be possible to provide even better counsel to patients as they consider risk reduction strategies.

Atypia was not included in the initial development of the Gail model due to a lack of pathologic assessment for all women in the Breast Cancer Detection and Demonstration Project. Thus, atypia was added to the original model using estimates of the population prevalence of atypia, and the relative risk for breast cancer associated with atypia. This modification was based on a prevalence of atypia of 7.8%, and relative risk for breast cancer of 1.96.<sup>6</sup> However, recent studies evaluating the risk of atypia, based on the more stringent criteria of Dupont and Page,<sup>8</sup> have all reported higher relative risks with atypia (3 to 5.3),<sup>1-5</sup> and lower prevalence (approximately 4%). This may explain the underestimates that we report here.

Another potential explanation of the observed underestimate is that the Gail model is intended as a prospective risk prediction tool, and accounts for death as a competing risk. This results in lower predicted probabilities. In our study, we used the Gail model to predict risk in women for whom outcomes, including death, had already been observed. To assess the degree to which the competing risk of death might have influenced the results of our comparison to the Gail model, we recomputed the Gail model risk probabilities while accounting for death as a competing risk in the most extreme way possible. That is, we considered women who died to still have been at

risk until they would have reached age 90 years. Even in this extreme case, the Gail model significantly underpredicted the number of breast cancer events (observed-to-expected ratio, 1.44; 95% CI, 1.11 to 1.8;  $P = .006$ ). However, this approach did result in a higher  $c$ -statistic (0.55; 95% CI, 0.49 to 0.60).

The Gail model has been studied in several settings; however, to our knowledge, data regarding atypia were not available in those validation studies.<sup>13,18-21</sup> In general, the model has fulfilled its original goal—to identify groups of at-risk women suitable for chemoprevention trials.<sup>5,13,22,23</sup> However, the model is increasingly used clinically to predict risk of individual women, and here the Gail model (and others) falls short of the precision required to make treatment recommendations for individual patients.<sup>5,13,22,23</sup> Better performance for population than an individual by these models is explained because the models were derived by averaging information across groups of individuals. When such models are based on large representative groups of patients, this leads to predictions that are well calibrated within an entire group, but do not guarantee accurate predictions for specific individuals within these groups.

To our knowledge, this article is the first report on the Gail model exclusively in women with atypia. It uses data from a large cohort defined by contemporary pathology review, with detailed risk factor information and long-term follow-up. It is limited predominantly by the small number of patients that developed breast cancer and use of data from open, rather than core, biopsy.

The Gail model uses demographic and clinical factors. It is possible that risk assessment could be improved through use of tissue-based risk factors, which should be feasible for all women who undergo a breast biopsy with benign findings (an estimated one million such women in the United States alone each year).<sup>24-26</sup> One of the hypotheses of breast cancer development is the existence of a continuum, wherein breast cells undergo successive alterations at a molecular level that lead from normal epithelium, to excess proliferation, and then to atypia, carcinoma in situ, and ultimately invasive carcinoma.<sup>2</sup> If this hypothesis is accurate, then tissue-based and molecular assessments that reflect the current state of the at-risk tissue will likely provide information leading to more accurate risk predictions. For example, in this atypia cohort the risk factors included in the Gail model do not stratify risk (Table 1). Presumably, the risk inherent in these factors (eg, family history) is already reflected in the tissue phenotype of atypia. We have recently shown that pathologic assessment of number of foci with atypia on biopsy stratifies risk of these patients.<sup>1</sup> We have also shown that the presence of lobular involution in background breast tissue and cyclo-oxygenase 2 overexpression further stratifies risk in women with atypia.<sup>9,28</sup> Additional work in groups of women with measurements of tissue-based biomarkers as well as breast cancer outcomes is likely to provide important information.

In summary, our findings suggest that Gail model risk estimates for our cohort of women with atypia are significantly lower than what was observed with long-term follow-up. At the level of the individual, there was low concordance between the Gail model predictions and actual breast cancer events. This study underscores the need for caution when using the Gail model to counsel individual women with atypia regarding their risk of developing invasive breast cancer. Additional research is required to identify highly predictive markers of breast cancer risk, and to incorporate these markers into a more accurate model for use in this high-risk population.

## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

## AUTHOR CONTRIBUTIONS

**Conception and design:** V. Shané Pankratz, Lynn C. Hartmann, Amy C. Degenim, Karthik Ghosh  
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A Novel Breast Tissue Feature Strongly Associated with Risk of Breast Cancer.  
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## ABSTRACT

Background: Accurate, individualized risk prediction for breast cancer is lacking. Tissue-based features may help to stratify women into different risk levels. Breast lobules are the anatomic sites of origin of breast cancer. As women age, these lobular structures should regress, which results in reduced breast cancer risk. But this does not occur in all women.

Methods: We have quantified the extent of lobule regression on a benign breast biopsy in 85 breast cancer cases and 142 age-matched controls from the Mayo Benign Breast Disease Cohort, by determining number of acini/lobule and lobular area. We also calculated Gail model 5-year predicted risks for these women.

Results: There is a step-wise increase in breast cancer risk with increasing numbers of acini/lobule ( $p=0.0004$ ). Adjusting for Gail model score, parity, histology and family history did not attenuate this association. Lobular area was similarly associated with risk. The Gail model estimates were associated with risk of breast cancer ( $p=0.03$ ). We examined the individual accuracy of these measures using the concordance (c) statistic. The Gail model c statistic was 0.60 (95% CI; 0.50-0.70); the acinar count c statistic was 0.65(95% CI; 0.54-0.75). Combining acinar count and lobular area, the c statistic was 0.68 (95% CI; 0.58-0.78). Adding the Gail model to these did not improve the c statistic.

Conclusion: Novel, tissue-based features that reflect the status of a woman's normal breast lobules are associated with breast cancer risk. These features may offer a novel strategy for risk prediction.

## INTRODUCTION:

The medical community's ability to predict risk of breast cancer for individual women is very limited.<sup>1-3</sup> In other tissues, optimal cancer risk prediction can occur when the tissue at risk is examined for evidence of premalignant change (e.g. cervix, esophagus, colon). Presumably the field of normal tissue, exposed to an individual's endogenous and exogenous risks, responds with a phenotype (e.g. proliferation, atypical cells) that reflects the increased risk. While breast tissue is not readily available for routine clinical assessment, women with benign breast disease have had breast tissue removed in the course of their care and have an increased risk of a later breast cancer.<sup>4-5</sup>

Current characterization of benign breast tissue focuses primarily on the degree and type of epithelial hyperplasia, but this focus may overlook other important, easily assessed features.<sup>4-6</sup> The breast is organized into 15-20 major lobes, each composed of lobules that contain the milk-forming acini. The lobule (or terminal duct lobular unit, TDLU) is the anatomic substructure thought to give rise to breast cancer.<sup>7</sup> Normal aging results in the physiologic regression (or involution) of breast lobules (Figure 1).<sup>8-11</sup> With regression, there is progressive loss of acini within the lobular units and replacement of specialized intralobular connective tissue with the collagen more typical of the interlobular region (Figure 1).<sup>8</sup> We previously showed that breast cancer risk decreases

with regression of lobular units, assessed qualitatively as no, partial, or complete involution.<sup>11</sup>

We hypothesized that a quantitative assessment of involution could be developed as a more precise and physiologic measure of breast cancer risk. Thus, in a nested-case control series within the Mayo Benign Breast Disease Cohort, we have calculated the number of acini within normal lobules and average lobule size. Here we show the risk prediction capabilities of lobule status and compare these results to the current standard, a Gail model assessment of risk performed in the same women.

#### **METHODS:**

##### *Study population*

We performed a nested-case control study within the Mayo Benign Breast Disease Cohort. This cohort includes all women (n=9,376) who had an open breast biopsy, with benign findings, at Mayo Clinic between 1-1-1967 and 12-31-1991.<sup>4,11</sup> Median follow-up for breast cancer events is 16.9 years.<sup>11</sup> For cohort members, we assembled risk factor and outcomes data from a study specific questionnaire and the Mayo Clinic medical record. Study pathologists characterized the benign biopsies, including the extent of lobule regression.<sup>11</sup> All patient contact materials and procedures were reviewed and approved by the Mayo Clinic Institutional Review Board.

For the current study, we selected a random sample of 100 cases who developed breast cancer from the cohort, stratified by five-year categories of year of benign biopsy to represent the entire spectrum of the cohort. We matched two controls to each case based on age and year of benign biopsy. Of these subjects selected, 85 cases and 142 controls had adequate tissue available for assessment.

##### *Assessment of lobular status*

Extent of lobular regression was previously characterized qualitatively by the study pathologist as none (0% TDLUs regressed); partial (1-74% regressed) or complete ( $\geq 75\%$  regressed).<sup>11</sup> For the quantitative assessments, one H & E stained slide per subject was scanned into the computer and analyzed using WebSlide Browser software (Bacus Labs product). This software allows the measurement of structural features (lobular area, acini number) as visualized by light microscopy (Figure 2).

The ten largest normal lobules were assessed for each patient by one observer (KPM) without knowledge of case status or previous pathologic assessment. If fewer than ten normal lobules were present, all were assessed. Analysis included (i) counting the number of individual acini per lobular unit and (ii) delineating the circumference of the lobule to measure its area in square microns (Figure 2). We defined countable acini as nuclei forming a distinct circular pattern with or without the presence of a discernible lumen. Distinct lobules were defined by the presence of intersecting stromal tissue. Abnormal lobules, namely those that contained large portions of terminal ducts, atypical lobular or ductal hyperplasia, sclerosing adenosis, large cysts, or proliferative disease without atypia were not included.

#### Reproducibility

A random sample of 82 slides (25 cases and 57 controls) was read by a second observer (JLC) using the quantitative, manual method described above. A different approach, automated analysis, was performed on another random sample of 95 slides (28 cases and 67 controls) using the Automated Cellular Imaging System (ACIS® III) instrument (Dako, Carpinteria, CA). The ACIS® III automatically scanned the study slides at 4x magnification to obtain an overall image. The images were then visually analyzed (by DM) to determine the 10 largest normal breast lobules (or less if there were not 10 lobules present on the slide). Area measurements were determined by tracing an outline of the lobules using the "free-form" tracing tool. Breast acini were counted within each lobule using the "100x" circle scoring tool. Area measurements and the number of acini were calculated by the instrument and exported from the ACIS® III program to a spreadsheet for statistical analysis.

#### Gail model calculations

Using age at benign biopsy as the age at risk assessment, the Gail model [NCI Breast Cancer Risk Assessment Tool, (<http://cancer.gov/bcrisktool>)] was used to predict the five-year risk of breast cancer for each of the women using their risk factor profile.<sup>12,13</sup> To calculate these estimates, we employed a FORTRAN program provided to us by the NCI (Gail M, Benichou J, Pee D [Information Management Services, Rockville, MD]: personal communication) which we have used previously.<sup>3</sup> This program contains the code that comprises the underlying calculation machinery used in the NCI's Breast Cancer Risk Assessment Tool (BCRAT). For variables with missing data, we used the standards in the online Gail model. To verify agreement between the code we used and the online tool, we randomly selected 10 subjects from our cohort and compared the 5-year and lifetime risk estimates obtained from the code given us to those from the online risk assessment tool.<sup>3</sup> All of the estimates were in complete agreement.

#### Statistical analyses:

We studied two measures of involution: the number of acini per lobule and lobule size. Primary analyses used the median of the values obtained across the multiple lobules measured for each woman. Secondary analyses that incorporated the values for all lobules were also performed, using repeated measures approaches, but results were similar to those modeling the medians and thus are not shown.

We compared distributions of number of acini and lobule area across demographic and clinical variables using general linear mixed models, accounting for the matched study design by fitting each case-control set as a random intercept term. Due to data skewness, analyses were run using log-transformed values. The resulting least squares means and 95% confidence intervals were then back-transformed to their original sampling units. We examined correlations between number of acini and lobule area; between these measures and our original three categories of involution (none, partial, complete); and between the quantitative measures obtained by the two manual observers and the ACIS method, using Pearson correlation coefficients, again based on the log-transformed values.

We assessed associations between number of acini, lobule area, and Gail model risk estimates with breast cancer risk using conditional logistic regression analysis. We first modeled each variable as categorical, pooling values into four to six distinct groups. We then assessed dose-response effects by fitting each as a continuous variable in the logistic model. These latter analyses were carried out using log-transformed values for acini and area, as assessments of their functional form revealed sub-optimal model fit using the data in its original scale. We examined univariate associations and models with various combinations of the following variables: the five-year Gail Model risk prediction score, number of live births, family history, and histologic findings. Using the risk estimates from the logistic models, we examined the risk prediction capabilities of these variables using concordance (c) statistics. These statistics can be interpreted as the area under the receiver operating characteristics (ROC) curve, or alternatively as the average sensitivity of the variable across all possible levels of specificity. We used a modified c statistic to account for the matched study design, calculating the number of case-control pairs in each set, as well as the number of "concordant" pairs (those for which the case's predicted risk exceeded the control's), then aggregating across all matched sets. 95% confidence intervals (CI) were calculated using 5000 bootstrap samples of case-control sets.

## RESULTS:

### Patient characteristics

A total of 227 patients were included in the nested case control-study: 85 women who went on to develop breast cancer (cases) and their 142 age-matched controls. The median follow-up for all participants was 16.2 years. The median follow-up for controls was 18.6 years; for cases, 9 years (follow-up ceases after breast cancer diagnosis). The mean age at benign biopsy was 52.1 years. The patient characteristics are provided in Table I.

### Number of acini per lobule (acinar count)

As expected, the average number of acini per lobule was associated with the pathologist's qualitative category of involution. Namely, women with no involution had a higher mean acinar count [32.0 (95% CI 26.4-38.8)] than women with partial involution [19.7 (95% CI 17.5-22.2)] or complete involution [7.7 (95% CI 5.8-10.3)] ( $p<0.0001$ ). When comparing the acinar count with histologic category [non-proliferative (NP), proliferative disease without atypia (PDWA), atypical hyperplasia (AH)], the means were not significantly different (18.8 [95% CI: 16.1-22.0], 22.1 [95% CI: 18.6-26.3], and 18.6 [95% CI: 13.2-26.2], respectively) ( $p=0.309$ ). The acinar count for women with a family history of breast cancer was 22.9 (95% CI: 19.0-27.6) vs. 18.3 (95% CI: 16.4-21.6) for those with no family history ( $p=0.0683$ ).

When comparing the acinar count for cases vs. controls, women who developed breast cancer had significantly more acini per lobule (24.3) than women who remained unaffected (17.8) ( $p=0.0008$ ). In Table 2 we show a step-wise increase in risk of breast cancer with increasing numbers of acini/lobule ( $p=0.0004$ ). This association was similar,

if not slightly stronger, after adjusting for the Gail model five-year risk score ( $p=0.0001$ , Table 2). We examined acinar count and breast cancer risk by nonproliferative vs. proliferative histologies and saw a similar dose response association in both groups (data not shown). Further adjustment for other potential confounders including parity and family history did not attenuate the observed association.

Because time from benign biopsy to breast cancer varied from 11 months to 27 years in our cases, we asked if involution measures varied by time to breast cancer. To investigate this, we plotted the ratio of involute in cases to involution in matched controls (on the log scale) as a function of time. We then fit a least squares regression line to this plot. The line had a slight downward trend but always remained above the back-transformed ratio values of 1.0, indicating that the positive association of acinar count with case status was sustained across the entire spectrum of time to cancer and did not vary significantly over time (data not shown).

#### Lobule size

Lobule area was strongly correlated with acinar count ( $r=0.85$ , 95% CI: 0.81-0.88). Women who developed breast cancer had a larger lobular area ( $64,165 \mu^2$ ) than controls ( $53,759 \mu^2$ ) ( $p=0.065$ ). Logistic regression analyses indicated a step-wise increase in risk of breast cancer with increasing lobule size ( $p=0.045$ ). Notably, during involution, acini become less cohesive geographically and can drift apart as seen in Figure 1B, resulting in a larger area than might be expected relative to number of acini. While lobule size was associated with breast cancer risk, associations were generally more modest than with number of acini.

#### Reproducibility

We compared the initial quantitative acinar count with those obtained by a second observer and with the automated ACIS readings. There was strong correlation among the three approaches (first and second observer,  $r=0.91$  [95% CI: 0.87-0.94]; first observer compared to ACIS,  $r=0.78$  [95% CI: 0.68-0.84]; second compared to ACIS,  $r=0.79$  [95% CI: 0.68-0.86]).

#### Gail model predictions

The Gail model 5-year estimates were associated with the outcome of breast cancer ( $p=0.030$ , Table 2B) for all breast cancer events -- invasive ( $n=69$ ), in situ ( $n=13$ ), and three with invasion status unknown. When restricting analyses to invasive cancers only, the Gail model results were very similar ( $p=0.022$ ).

#### Accuracy of risk prediction: Lobule measures vs. Gail model

We assessed the accuracy of risk prediction, for individual women, for the Gail model and for acinar count and lobular area, using the c statistic (Table 3). For the Gail model, it was similar to estimates found in other studies (0.60 [95% CI: 0.50-0.70]).<sup>14,15</sup> Using acinar count alone, the c statistic was 0.65 (95% CI: 0.54-0.75). Combining acinar count and lobular area increased the c statistic to 0.68 (95% CI: 0.58-0.78). Adding the Gail model to this combined set did not add to predictive accuracy (c statistic, 0.66).

## DISCUSSION:

Optimal early detection and prevention strategies for breast cancer require accurate identification of those individuals at significantly increased risk for the disease. Despite our knowledge of many determinants of breast cancer risk, both endogenous and exogenous, our ability to predict risk for individual women remains limited.<sup>1,2</sup> Reasoning that a woman's breast tissue reflects the integration of her exposures to multiple risk-contributing processes, we are working to develop a tissue-based approach to risk prediction for breast cancer. Regression, or involution, of lobules is a physiologic process that occurs as a woman ages.<sup>3,9</sup> Importantly, it is these same structures that give rise to breast cancer.<sup>7</sup> Completion of the involution process, assessed in a qualitative manner, is associated with a significant reduction in breast cancer risk.<sup>11</sup> We have now quantified extent of lobular regression for individual women via the number of acini/lobule and lobule size and show a strong association with risk of breast cancer. Importantly, these lobular features, assessed on a single H&E stained slide, identified those women who would later develop breast cancer more precisely than a Gail model prediction. This held true whether or not comparisons were restricted to invasive events. We have also shown reproducibility of these measures, whether obtained manually or in an automated fashion, with correlation coefficients of 0.78-0.91. Of note, these measures appear to be independent of histology, contributing to their risk prediction capabilities.<sup>11</sup>

Several risk prediction models for breast cancer focus on an individual's likelihood of carrying a hereditary predisposition to the disease.<sup>16-22</sup> Outside the hereditary setting, the most widely used tool is the Gail model.<sup>12</sup> This model is available on the National Cancer Institute's website ([www.cancer.gov/brisktool/](http://www.cancer.gov/brisktool/)) and is viewed approximately 20,000-30,000 times a month,<sup>2</sup> demonstrating the strong clinical demand for risk assessment for individual women. While the Gail model has been shown to be well-calibrated in predicting the number of invasive cancers likely to develop in groups of women, its discriminatory accuracy in predicting risk for individual women, as measured by c statistics near or below 0.6, is only slightly better than chance alone. Here we show that a simple physiologic measure of lobular status is more strongly associated with breast cancer risk than the Gail model.

There are several plausible mechanisms by which progressive degrees of lobular involution may reduce breast cancer risk. The most straightforward is that the dramatic reduction in epithelial cell number that occurs with involution equates to a physiologic "prophylactic mastectomy."<sup>23</sup> This can be visualized in Figure 3 where age-related lobular involution has essentially removed the TDLUs from the field of breast tissue. Another explanation is that age-related involution invokes some final differentiation-sensescence program, rendering the remaining cells resistant to carcinogenic influence. It is somewhat counterintuitive that an age-related process like involution is associated with reduced breast cancer risk, when breast cancer risk increases with age. Notably, in studying all women over 55 in our cohort, those who had complete involution had a RR for breast cancer of 0.92 (95% CI: 0.74-1.14) vs. 3.21 (95% CI: 1.90-5.08) for those with no involution.<sup>11</sup> This suggests that age-related breast cancer risk may be concentrated in women whose lobules fail to regress normally.

Our study has several limitations. First, these findings do not necessarily pertain to all women because our cohort includes women who had a breast biopsy for some concern. Moreover, the present study is based in a nested case-control study from our larger Mayo Benign Breast Disease Cohort. However, we randomly selected this sample from the entire set of cases and our previous results, based in the entire cohort, showed that involution status assessed qualitatively (none, partial, complete) was strongly associated with breast cancer risk.<sup>11</sup> Even if our findings are limited to women with benign breast disease, such women number at least one million per year in the United States alone,<sup>24-26</sup> and they represent a clinically important group, as about 25% of women with breast cancer have had a prior benign biopsy.<sup>27</sup> A limitation in our comparisons to the Gail model is that our controls were matched to cases on age at benign biopsy. Since age is one of the predictor variables in the Gail model, this matching may limit the risk prediction capabilities of the Gail model. Analysis of only one slide per woman could be a limitation. However, we have looked at uniformity of involution across the field of a woman's breast tissue in women who had bilateral prophylactic mastectomy and have demonstrated high concordance in involution status across all eight quadrants of their breast tissue.<sup>28</sup> Importantly, our analyses are based on a modest sample size. Although we found statistically significant associations between acinar count and breast cancer risk, confidence intervals are wide. Further studies are needed to confirm our results.

In summary, we have developed a means to assess degree of regression of normal breast lobules quantitatively. We have shown that higher acinar counts within the lobules, and larger lobule size, are associated with higher risk of breast cancer. These simple physiologic features may offer an alternative strategy for breast cancer risk prediction in women who have had benign breast biopsies.

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Lynn C. Hartman, M.D., had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

The authors have no conflicts of interest.

**Figure legends**

In Figure 1 panel A, there is a field of normal lobules (terminal duct lobular units), each composed of multiple acini. In panel B there has been complete regression (involution) of these lobules leaving small residual structures largely depleted of acini.

In Figure 2 panel A, we subdivide an intact lobule to facilitate counting of individual acini. Panel B demonstrates the delineation of the circumference of the lobule for calculation of lobule area by the computer software.

In Figure 3, whole breast mounts from pre-involutional (A) and post-involutional women. (With kind permission of Springer Science+Business Media. Originally published in "Handbuch der mikroskopischen Anatomie des Menschen." (W. Bargmann, ed.), Vol 3, part 3, Haut und Sinnesorgane, pp. 277-485, 1957. Springer-Verlag, Berlin)

Table 1. Clinical and Histologic Characteristics

Attribute	Cases (N=85)	Controls (N=142)	Total (N=227)
Age at Biopsy			
Mean (SD)	52.5 (10.1)	51.8 (10.1)	52.1 (10.1)
Histologic Type, N (%)			
Non-proliferative	42 (49.4%)	79 (55.6%)	121 (53.3%)
Proliferative without atypia	31 (36.5%)	56 (39.4%)	87 (38.3%)
Proliferative with atypia	12 (14.1%)	7 (4.9%)	19 (8.4%)
Number of live births, N (%)			
Missing	0 (0%)	1 (0.7%)	1 (0.4%)
Nulliparous	11 (12.9%)	18 (12.7%)	29 (12.8%)
1	7 (8.2%)	15 (10.6%)	22 (9.7%)
2	30 (35.3%)	38 (26.8%)	68 (30%)
3	26 (30.6%)	29 (20.4%)	55 (24.2%)
4+	11 (12.9%)	41 (28.9%)	52 (22.9%)
Family History of Breast Cancer, N (%)			
Missing	1 (1.2%)	5 (3.5%)	6 (2.6%)
None	51 (60.0%)	96 (67.6%)	147 (64.8%)
Weak	16 (18.8%)	22 (15.5%)	38 (16.7%)
Strong	17 (20.0%)	19 (13.4%)	36 (15.9%)

Table 2. Association of acini count and Gail model with risk of breast cancer (invasive and in situ)

A. Number of acini/ lobule	Cases	Controls	RR	P-value <sup>2</sup>	RR (95% CI) <sup>1,3</sup>	P-value <sup>3</sup>	B. Gail 5- year risk	Cases	Controls	RR	P-value	RR (95% CI) <sup>1,4</sup>	P-value <sup>4</sup>
			(95% CI) <sup>1,2</sup>							(95% CI) <sup>1,2</sup>		(95% CI) <sup>1,4</sup>	
0-10	16	37	1.00 (REF)	0.0004	1.00 (REF)	0.0001	0-≤1	12	33	1.00 (REF)	0.030	1.00 (REF)	0.037
11-20	22	41	2.80 (0.88 - 8.93)		3.61 (1.03-12.63)		1-≤2	38	72	1.76 (0.64 - 4.87)		1.58 (0.53 - 4.73)	
21-30	13	26	3.23 (0.87 - 11.94)		4.77 (1.14-19.99)		2-≤3	19	38	4.13 (1.28 - 13.32)		3.83 (1.09 - 13.47)	
31-40	8	18	3.23 (0.72 - 14.41)		3.95 (0.81-19.36)		3+	16	19	3.55 (1.05 - 11.98)		3.51 (0.92 - 13.35)	
41 or more	26	20	11.85 (2.92 - 48.12)		14.03 (3.18-61.86)								

1. Relative risks and 95% confidence intervals calculated using conditional logistic regression analysis. Analyses account for the matched nature of the data by modeling set ID as a stratification variable. P-values assess the dose-response effects of quantitative involution and the Gail model estimate with risk of breast cancer by modeling each as a continuously distributed predictor variable.

2. Univariate analyses

3. Multivariate analyses, adjusting additionally for the five-year predicted risk of breast cancer based on the Gail model.

4. Multivariate analyses, adjusting additionally for the number of acini/lobule.

Table 3. Assessment of the predictive capability of lobular measures and the Gail model using concordance statistics.

Predictors	c statistic (95% CI) <sup>1,2</sup>	c statistic (95% CI) <sup>1,3</sup>
	All cases (n=85)	Invasive only (n=69)
Gail model	0.60 (0.50 - 0.70)	0.59 (0.47 - 0.71)
Number of acini	0.65 (0.54 - 0.75)	0.69 (0.57 - 0.80)
Lobular area	0.56 (0.47 - 0.67)	0.59 (0.47 - 0.70)
Number of acini + area	0.68 (0.58 - 0.78)	0.70 (0.59 - 0.81)
Number of acini + area + Gail model	0.66 (0.56 - 0.76)	0.69 (0.58 - 0.80)

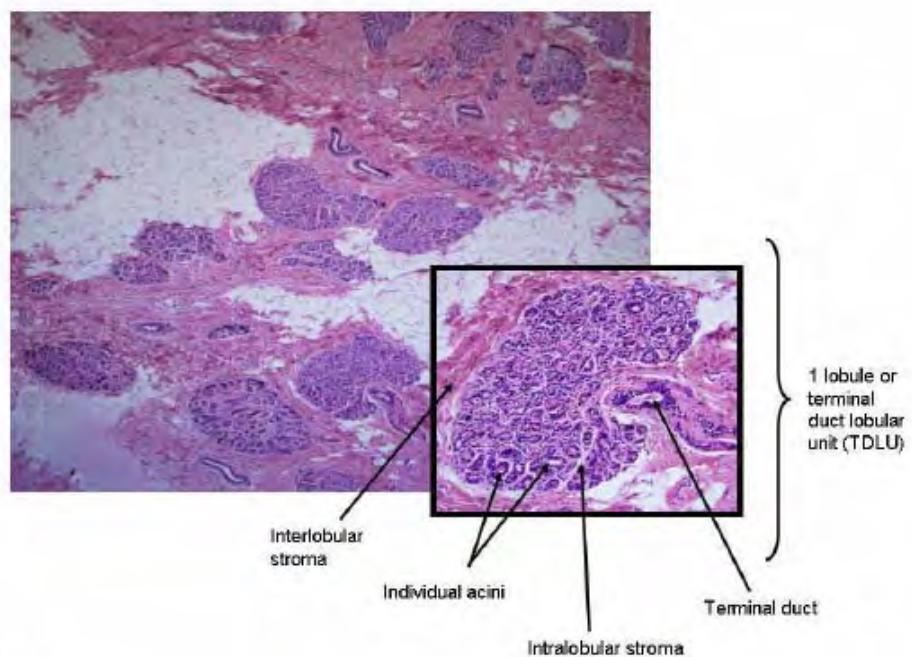
<sup>1</sup>Statistics calculated using conditional logistic regression analysis, accounting for the matched nature of the data by modeling set ID as a stratification variable.

<sup>2</sup>c statistic based on both invasive and in situ events

<sup>3</sup>c statistic based on invasive cases only

Figure 1.

(a)



(b)

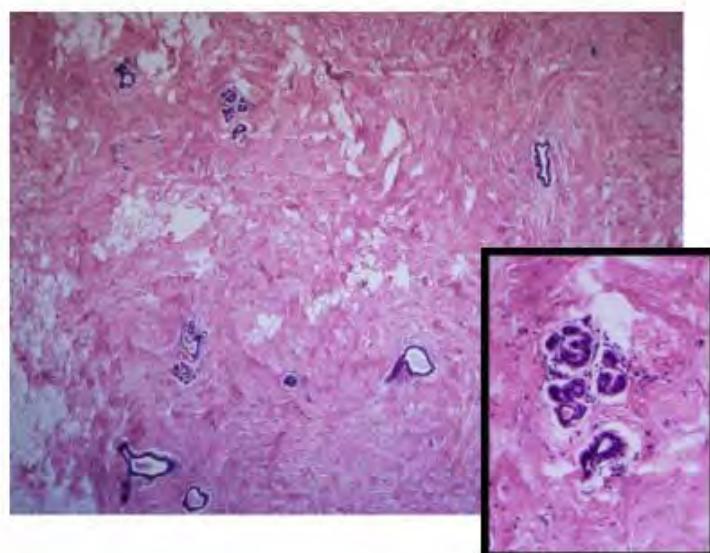
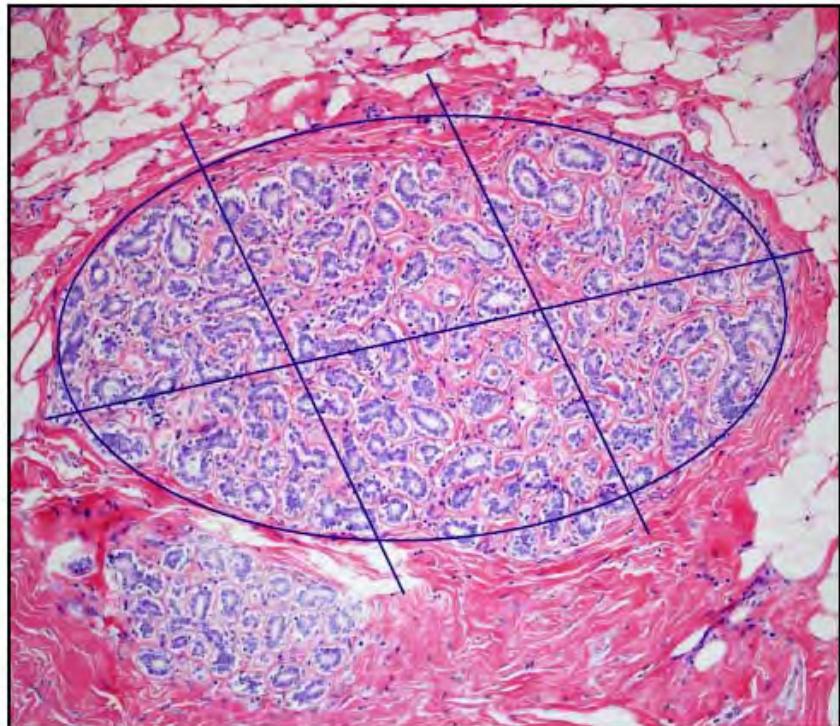


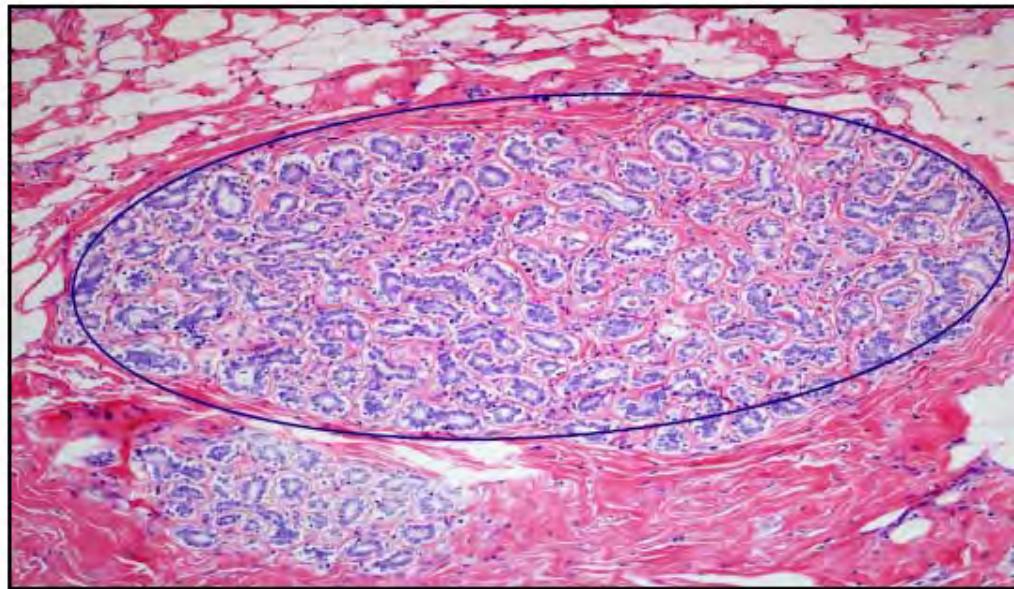
Figure 2

Panel A



**Number of Acini per**

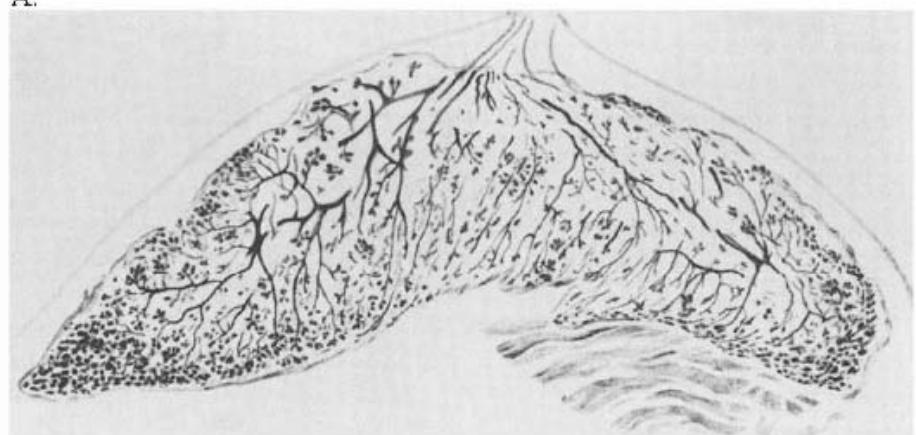
Panel B



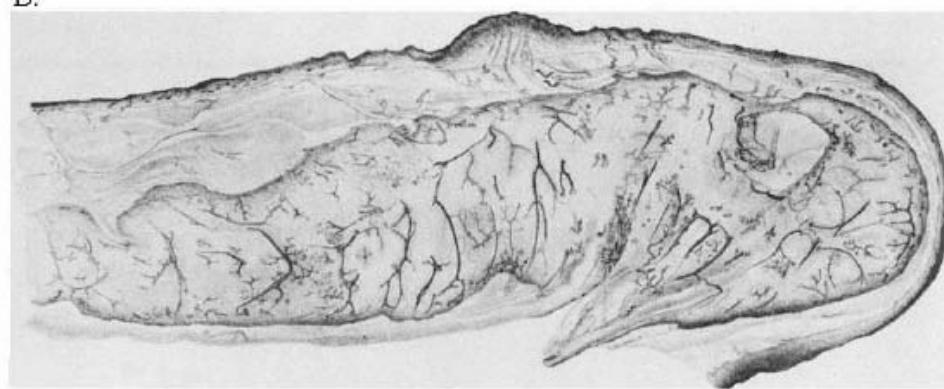
**Lobular Area in  $\mu^2$**

Figure 3

A.



B.



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